The European network for cell migration studies
www.incem.rwth-aachen.de

forces materials cellular responses

RWTH AACHEN UNIVERSITY

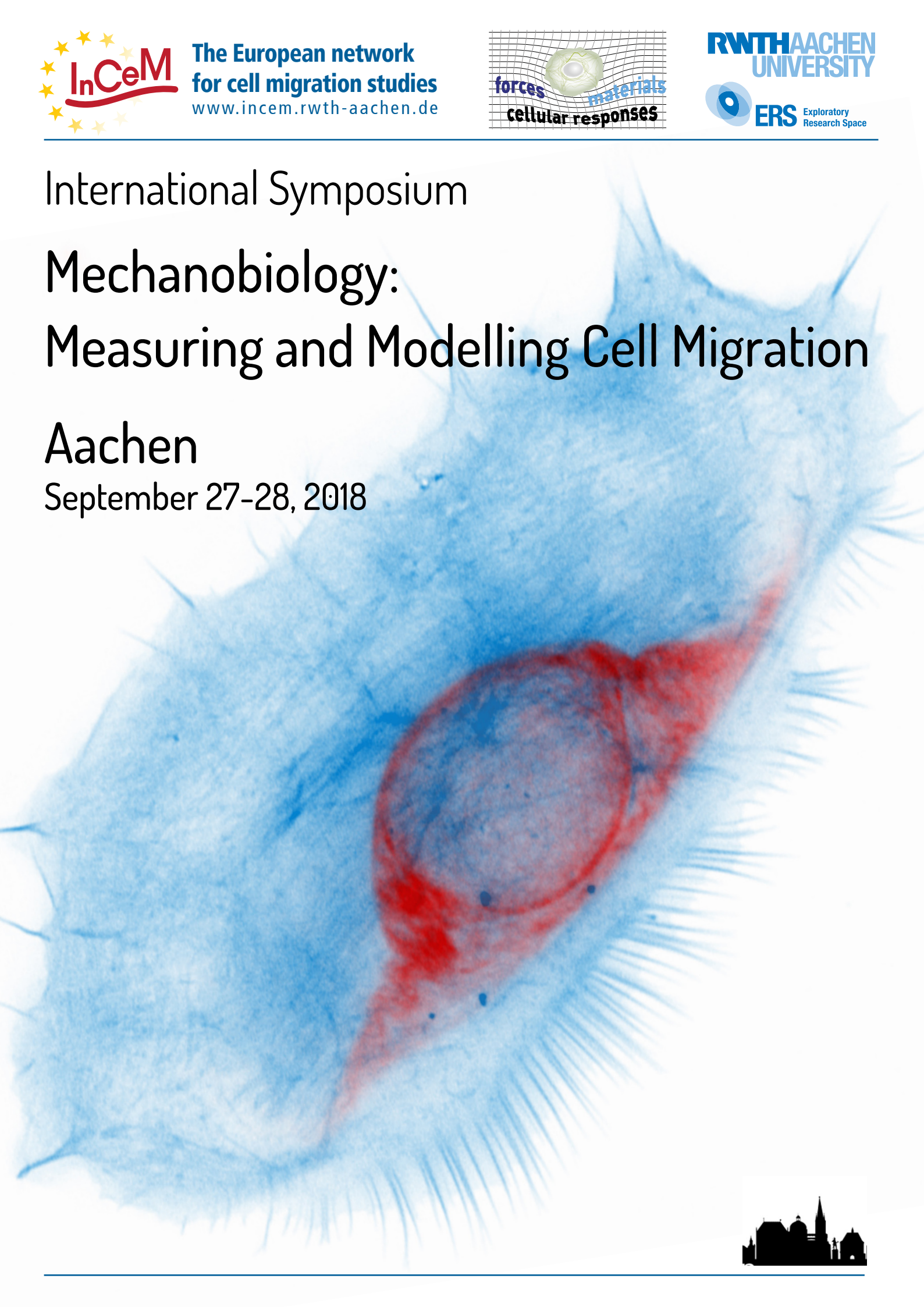
ERS Exploratory Research Space

International Symposium

Mechanobiology: Measuring and Modelling Cell Migration

Aachen

September 27-28, 2018



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The European network for cell migration studies

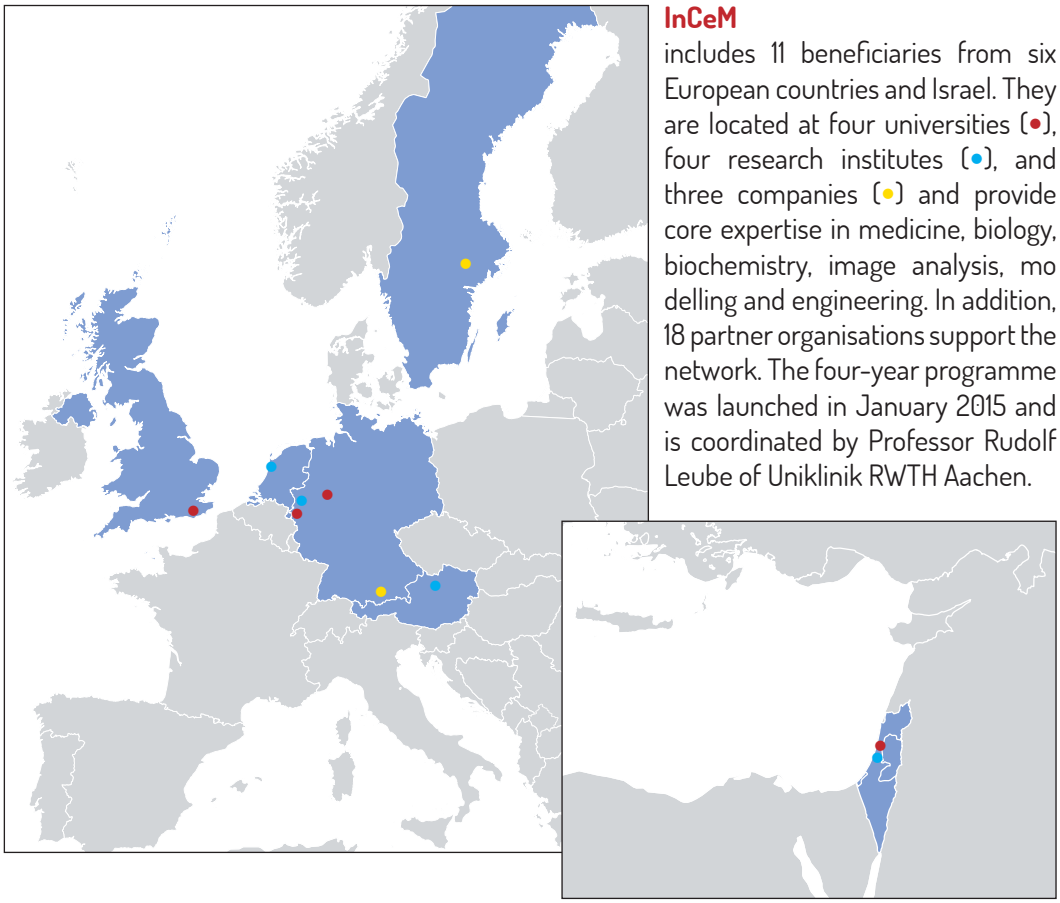
InCeM – Research Training Network on Integrated Component Cycling in Epithelial Cell Motility

InCeM is an international PhD programme for highly motivated young scientists, offering early-stage researchers the opportunity to improve their research and entrepreneurial skills and enhance their career prospects. This Marie Skłodowska-Curie Innovative Training Network is funded by the European Community's Framework programme HORIZON 2020. InCeM provides an international and highly interdisciplinary framework of collaborators from academia and industry with core expertise in medicine, biology, biochemistry, image analysis, modelling and engineering.

InCeM's primary research focus of is cell migration, a fundamental biological process occurring throughout the human body at any point in life. It is essential for vital processes such as tissue formation and wound healing and drives tissue invasion during carcinogenesis. Understanding and controlling cell migration will have major clinical impact.

InCeM's scientific goals are

- to develop and apply state-of-the-art experimental techniques that can visualise morphological, biochemical and physical processes of cell motility at different time and length scales in parallel,
- to integrate experimental data in advanced multi-scale models to establish fact-based and data-driven concepts of cell migration, and
- to translate the resulting insights into approaches to deliberately tune motile behaviour in relation to disease.



InCeM includes 11 beneficiaries from six European countries and Israel. They are located at four universities (•), four research institutes (•), and three companies (•) and provide core expertise in medicine, biology, biochemistry, image analysis, mo delling and engineering. In addition, 18 partner organisations support the network. The four-year programme was launched in January 2015 and is coordinated by Professor Rudolf Leube of Uniklinik RWTH Aachen.

The European network for cell migration studies

InCeM's objectives

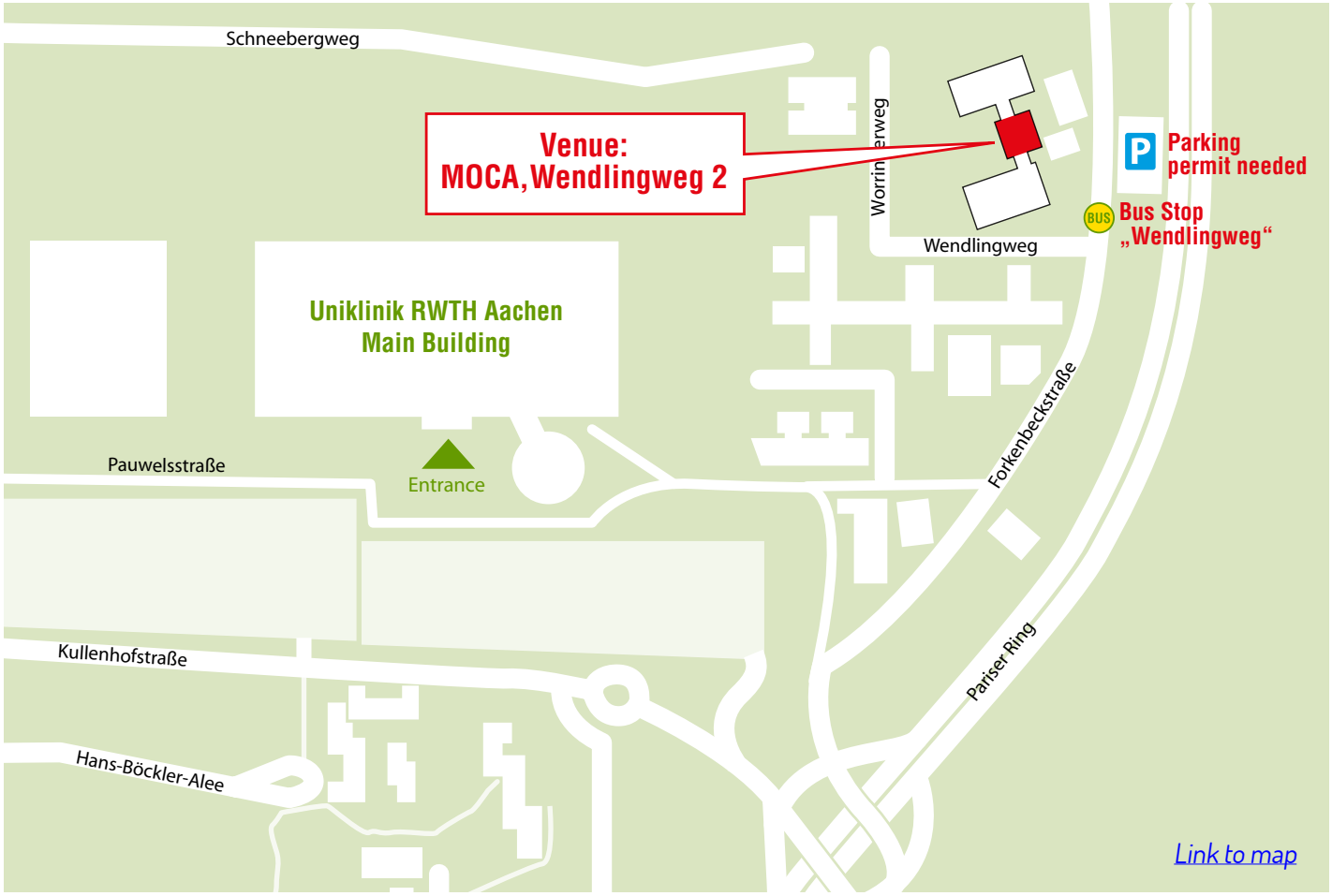
InCeM's main aim is to establish a joint research-training programme supported by an interdisciplinary and intersectoral network to provide early-stage researchers with deep insights into cutting-edge cell biology and modelling. The project will ensure Europe-wide exchanges to cater for mobility in today's globalised society. The integration of full industry partners and clinical advisors in the network offers ideal conditions for application-driven research.

The network will develop, strengthen and structure initial training of researchers at the European level based on a combination of local education programmes and adjunct institutions. The aim is to create an inspiring and productive environment on an international competitive level for the training of a new generation of highly educated researchers who will significantly contribute to the design of trials and experiments, the analysis of complex and multimodal data sets and knowledge translation.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 642866.

https://incem.rwth-aachen.de/

How to get there?



By Public Transport

Trainstation „Aachen HBF" (main station)

Take bus line 3A (direction Kaiserplatz×Ponttor×Uniklinik) at the bus stop immediately in front of the main station, or

3B (direction Schanz-Uniklinik-Ponttor) at the bus stop on the opposite side of the street in front of the main station

Exit at bus stop „Wendlingweg“

Trainstation „Aachen West“

At train station „Aachen West“ leave the platform downstairs and turn left towards the bus stop, do not cross the street

Bus lines 3A, 33, 73 und 173 (direction Uniklinik or Vaals, respectively), exit at the bus stop „Wendlingweg“

By Car: Parking

is possible at the car park "Forckenbeckstr." opposite to MOCA.
A permit is needed which you can get at InCem@rwth-aachen.de.

Registration and Badge Pickup

Registration:

Thursday, September 27th, 2018:
12:00 to 13:00

MOCA, Institute of Molecular and Cellular Anatomy, Medical Faculty
Wendlingweg 2
RWTH Aachen University
D-52057 Aachen

Scientific Organizing Committee MMCM 2018

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Meals And Refreshments

The following meals and refreshments are provided free of charge to attendees.

Thursday, September 27th, 2018

12:00 to 13:00 – Lunch
Foyer of auditorium

15:00 to 17:00 – Coffee Break and Poster Session
Foyer of auditorium

19:45 to 23:30 – Conference Dinner
Drehturm Aachen
Belvedereallee 5,
52070 Aachen

Friday, September 28th, 2018

10:45 to 11:15 – Coffee Break and Group Photo
Foyer of auditorium

12:45 to 14:00 – Farewell Snack
Foyer of auditorium

Internet Access

WiFi Guest-Access

Login:	MMCM-Aachen
Password:	cytubo
Validity:	2018.09.27 - 2018.09.28

Login:

- Connect to WiFi network "mops"

then:

- Scan this QR-Code

OR

- Visit any website (e.g. example.net)
- You will be redirected to the guest login
- Login in with above credentials

Scan QR-Code for automatic login when connected to mops

Eduroam

The Eduroam network is also available.

Note:

Guest network access is intended for scientific use only. Usage will be monitored and may be terminated at any time. The connection is unencrypted. Do not send any passwords or personal information over an unencrypted protocol. If possible use only HTTPS websites.

Keynote Speakers

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http://bement.molbio.wisc.edu/

Julie Plastino
Institut Curie
Biomimetism of Cellular Movement
Paris
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http://www.goetzlab.com/

Núria Gavara
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Program

Mechanobiology: Measuring and Modelling Cell Migration 2018

Thursday, September 27th

12:00-13:00	**Registration** MOCA, Institute of Molecular and Cellular Anatomy, Medical Faculty Wendlingweg 2 RWTH Aachen University D-52057 Aachen		
12:00-13:00	Snack		
13:00-13:15	Welcome (Coordinator)		
Part 1: Cell contraction and invasion **Chair: Galiya Sakaeva / Co-Chair: Rutuja Patwardhan**			
13:15-14:00	**Keynote lecture 1**	**Bill BEMENT** The cell cortex as an excitable medium	**Abstract K1**
14:00-14:07	**Flash talks 1-4**	**Galiya Sakaeva** Polarized epithelial cell migration depends on rear end stress fibre tension	**Abstract 01**
14:07-14:14		**Anne Pora** Focal adhesion-hemidesmosome crosstalk in migrating keratinocytes	**Abstract 02**
14:14-14:21		**Rutuja Patwardhan** EGF triggers Rho excitability in migrating keratinocytes	**Abstract 03**
14:21-14:28		**Victor Juma** Parameter identification for Rho-myosin model	**Abstract 04**
14:30-15:00	**The mentor's lecture**	**Leif DEHMELT** Self-organization of cell contraction dynamics	**Abstract M1**
15:00-17:00	**Poster session and coffee break**		
17:00-17:45	**Keynote lecture 2**	**Julie PLASTINO** Forces drive basement membrane invasion in *Caenorhabditis elegans*	**Abstract K2**

Program

17:45-17:52	**Flash talks 5-10**	**Jacopo di Russo** Collective cell migration: intercellular forces coordination by integrin α5β1	**Abstract 05**
17:52-17:59		**Aljona Gaiko-Shcherbak** The basement membrane: A mechanical cell invasion barrier in breast cancer	**Abstract 06**
17:59-18:06		**Volker Buck** *In vitro* models for studying endometrial receptivity – characterization of human biopsies in a new test system	**Abstract 07**
18:06-18:13		**Andreas Ludwig / Aaron Babendreyer** Regulation of fractalkine-induced monocytic cell recruitment by endothelial shear stress	**Abstract 08**
18:13-18:20		**Aldo Leal-Egana** Novel strategy to determine cancer cell heterogeneity *in vitro*: assaying the relation force/speed/morphology with micro-featured scaffolds	**Abstract 09**
18:20-18:27		**Jannis Körner** β1 subunit stabilises sodium channel Nav1.7 against mechanical stress	**Abstract 10**
18:30-19:15	**Keynote lecture 3**	**Jacky GOETZ, Sébastien HARLEPP** Biomechanical regulation of tumor metastasis	**Abstract K3**
19:30-19:45	**Transfer by bus**		
19:45-23:30	**Conference dinner**	**Drehturm Aachen** Belvedereallee 5, 52070 Aachen	

Friday, September 28th

Part 2: Cell mechanics as a basis for biomedical engineering

Chair: Rudolf Merkel / Co-Chair: Rupert Ecker

09:00-09:30	**The mentor's lecture**	**Michael KOZLOV** Modelling membrane super-structures formed by caveolae	**Abstract M2**

Program

09:30-09:37	**Flash talks 11-14**	**Roger Sauer** Advanced continuum modelling of lipid bilayer membranes	**Abstract 11**
09:37-09:44		**Davide Cusseddu** A coupled bulk-surface model for cell polarisation	**Abstract 12**
09:44-09:51		**Nadieh Kuijpers** Dynamic interaction of cytoskeletal filaments in migrating keratinocytes	**Abstract 13**
09:51-09:58		**Dmytro Kotsur** Automatic segmentation and tracking of keratin filaments in living cells	**Abstract 14**
10:00-10:45	**Keynote lecture 4**	**Núria GAVARA** The cytoskeleton, by the numbers	**Abstract K4**
10:45-11:15	**Coffee break and group photo**		
11:15-11:22	**Flash talks 15-20**	**Saransh Vora** A computational framework for simulating the dynamics of keratin filament networks	**Abstract 15**
11:22-11:29		**Eduard Campillo-Funollet** A Bayesian approach to parameter identification: keratin dynamics and traction force microscopy	**Abstract 16**
11:29-11:36		**Clara Abaurrea Velasco** Active composite agents: a minimal model for motile cells	**Abstract 17**
11:36-11:43		**Nikos Fatsis-Kavalopoulos** Precise microfluidic clustering of multiple cell types for quantifying paracrine effects in cancer cells	**Abstract 18**
11:43-11:50		**Rupert Ecker** TissueFAXS Cytometry – A Tool for Precision Medicine: Automated Analysis of Single Cells and the Cellular Microenvironment	**Abstract 19**
11:50-11:57		**Laura Bornes** Keratinocytes migrate individually in a collectively migrating sheet during wound closure *in vivo*	**Abstract 20**
12:00-12:30	**The mentor's lecture**	**Stefan JOCKENHÖVEL** Biohybrid implant development – The role of mechanobiological stimuli	**Abstract M4**
12:30-12:45	**Closing of the meeting**	Coordinators	
12:45-14:00	**Farewell snack**		

Posters

Abstracts
Keynote Lectures

Abstract – Keynote Lecture 1

The cell cortex as an excitable medium

Ani Varjabedian, Adriana Golding, Zac Swider, Ann Miller, Andrew Goryachev, and **Bill Bement**

Laboratory of Cell and Molecular Biology, University of Wisconsin-Madison, Madison-WI

Animal cell locomotion, cell repair, and cell division (cytokinesis) entail dynamic, patterned activation of Rho class GTPases at the cell cortex. The GTPases, in turn, direct the dynamic patterning of F-actin, myosin-2 via their various regulators. Traditionally, the regulation and dynamics of Rho GTPases and the cortical cytoskeleton during cell locomotion, cell repair, and cell division have been considered to be completely different, with locomotion being governed by external cues provided by chemoattractants, repair being governed by cues provided by cell damage, and cytokinesis being governed by internal cues provided by the spindle. Here we demonstrate that Rho GTPase activation/inactivation and cytoskeletal assembly/ disassembly and cytoskeletal are common features of these three processes. We further show that during cytokinesis the cell cortex behaves as an excitable medium, implying that it has the potential to not only receive but to interpret and transmit signals. Because locomoting cells likewise show properties of excitable media, we propose that cortical excitability is a fundamental property that endows cells with many of those features associated with life.

Abstract - Keynote Lecture 2

Forces drive basement membrane invasion in *Caenorhabditis elegans*

Julie PLASTINO

Institut Curie, Biomimetism of Cellular Movement, Paris

During invasion cells breach basement membrane (BM) barriers with actin-rich protrusions. It remains unclear, however, if actin polymerization applies pushing forces to help break through BM, or if actin filaments play a passive role as scaffolding for targeting invasive machinery. Here using the developmental event of anchor cell (AC) invasion in *Caenorhabditis elegans*, we observe that the AC deforms the BM just prior to invasion, exerting forces in the tens of nN range. BM deformation is driven by actin polymerization nucleated by the Arp2/3 complex and its activators, while formins and crosslinkers are dispensable. Delays in invasion upon actin regulator loss are not caused by defects in AC polarity, trafficking or secretion, as appropriate markers are correctly localized in the AC even when actin is reduced and invasion is disrupted. Overall force production emerges from this study as the central tool that invading cells use to promote BM disruption.

Abstract - Keynote Lecture 3

Tracking tumor metastasis in vivo at high spatio-temporal resolution

Gautier Follain, Nael Osmani, Sofia Azevedo, Luc Mercier, Olivier Lefebvre, **Sébastien Harlepp**, Jacky G. Goetz

INSERM U1109, Goetz Lab Tumor Biomechanics, Université de Strasbourg, STRASBOURG

Cancer progression is a complex process in which an invasive primary tumor spreads to distant organs to form life-threatening metastases. While evidences suggest that metastatic seeding is driven by cell-intrinsic and environmental cues, contribution of mechanical cues such as vascular architecture and hemodynamic has been poorly studied. We aim to elucidate the impact of blood flow on arrest, adhesion and extravasation of circulating tumor cells in vivo, combining high-speed imaging, correlative light and electron microscopy (CLEM) and biophysical tools. We exploit the advantages of Zebrafish embryos where hemodynamic profiles can be tuned using pharmacological approaches and microfluidic set up. First, using live-injection of tumor cells, we showed that arrest of CTCs occurs mostly in vessels with favorable flow profiles controlling the adhesion of CTCs to the endothelium. We identified the threshold values of flow and adhesion forces allowing arrest of CTCs with optical tweezers both in vitro and in vivo. Second, using quantitative mapping of extravasation, we observed that low flow forces drastically impaired tumor cell extravasation. Thanks to time lapse imaging and intravital CLEM approach, we elucidated that flow forces are required for the remodeling properties of the endothelial cells next to arrested tumor cells. So doing, we described a new mechanism of tumor extravasation in vivo. Importantly, we also observed endothelial remodeling at arrest sites of CTCs in mouse brain capillaries. Finally, we established a significant correlation comparing human brain perfusion map (MRI) with metastatic sites in the brain of 100 patients, confirming the relevance of our results. Altogether, we demonstrated that hemodynamic profiles at metastatic sites regulate extravasation and subsequent outgrowth.

Abstract - Keynote Lecture 4

The cytoskeleton, by the numbers

Núria GAVARA

Queen Mary University of London, School of Engineering and Materials Science

The cell's cytoskeleton is a fascinating and dynamic structure, both from a biophysical and a biomedical perspective. On the one hand, the cytoskeleton confers mechanical stability to the cell, being composed of 3 polymeric networks that each excel at a particular mechanical role: supporting tension (actin), compression (microtubulues) or strain (intermediate filaments). On the other hand, the cytoskeleton is crucial for cell function (such as migration or division), regulates cell fate, and is involved in a large number of pathologies including cancer and inflammation. Under the fluorescence microscope, the cytoskeleton is a striking structure that perfectly captures the concept of 'form follows function'. Taking advantage of this, we have developed advanced image analysis pipelines (CSK morphometrics) that allow us to quantify in a multiparametric approach the amount and intracellular organization of cytoskeletal networks. We use this high-throughput multiplex approach, for example, to better understand the mechanical interplay between the cytoskeleton and the nucleus, and how this can affect nuclear and chromatin states. In addition, when coupled to machine learning algorithms, we use CSK morphometrics for cellular age diagnostics or to monitor cell fate in differentiating stem cells. Finally, when used simultaneously with other biophysical tools, such as Atomic Force Microscopy, we can further characterize the role of cytoskeletal organization in the mechanical properties of adherent cells.

Abstracts
The Mentor's Lectures

Abstract - The Mentor's Lecture 1

Self-organization of cell contraction dynamics

Dominic Kamps[1,2], Johannes Koch[3], Victor Yuma[4], Rutuja Patwardhan[3], Eduard Campillo-Funollet[4], Melanie Graessl[3], Soumya Banerjee[1,2], Tomáš Mazel[1,2], Aneta Koseska[1], Malte Schmick[1], Xi Chen[5], Yaowen Wu[5], Stephanie Portret[6], Anotida Madzvamuse[4], Perihan Nalbant[3], Leif Dehmelt[1,2]

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[3]Department of Molecular Cell Biology, Center for Medical Biotechnology, University of Duisburg-Essen, 45141 Essen, Germany

[4]Department of Mathematics, Pevensey III, BN1 9QH, Brighton, UK

[5]Chemical Genomics Centre of the Max-Planck Society, Dortmund, Germany

[6]Department of Mathematics, University of Manitoba, Winnipeg, R3T 2N2, Canada

Summary: Pulsatile, local contractile forces play important roles during embryonic development. Our recent studies revealed that such contraction pulses emerge from a combination of positive and negative feedback in a Rho/myosin-based signal network, and that this dynamic behavior is modulated by mechanical properties of the cellular environment[1]. Here we used photochemistry and optogenetics to apply direct, acute, experimental manipulations of critical components of this signal network to study their causal relationships. We used this experimental data to derive a biochemical model and to fit model parameters to perturbation-response kinetics and cross correlation functions. Analysis of system dynamics predicted switching between stable and oscillatory states and maximal pulse amplitude and regularity at intermediate concentrations of the positive feedback mediator GEF-H1. These predictions were confirmed by experimental manipulations of the effective concentration of GEF-H1 in cells. Furthermore, an extended theoretical concept that includes diffusion of the active and inactive system components can explain spatially focused cell contractility pulses and recurring wave propagation by an additional, long-range negative feedback via activator depletion. Our study offers a theoretical framework that is based on detailed experimental analysis to describe a reaction-diffusion system that locally focuses cell contractility signals. We propose that subcellular cell contraction pulses that emerge from this reaction-diffusion system enable an active, exploratory process that locally probes the elasticity of the extracellular environment.

Reference:

[1]Graessl M, Koch J, Calderon A, Kamps D, Banerjee S, Mazel T, Schulze N, Jungkurth JK, Patwardhan R, Solouk D, Hampe N, Hoffmann B, Dehmelt L, Nalbant P. An excitable Rho GTPase signaling network generates dynamic subcellular contraction patterns. *J Cell Biol.* (2017) **216**:4271

Abstract - The Mentor's Lecture 2

MODELING MEMBRANE SUPER-STRUCTURES FORMED BY CAVEOLAE

Michael KOZLOV

Tel Aviv University, Department of Physiology and Pharmacology, Sackler School of Medicine

Caveolae, the flask-shaped pits covered by caveolin-cavin coats, are abundant features of the plasma membrane of many cells. Besides appearing as single membrane indentations, caveolae are organized as superstructures in the form of rosette-like clusters, whose mechanism of assembly and biological functions have been elusive. Here we propose that clustering of caveolae is driven by forces originating from the elastic energy of membrane bending deformations and membrane tension. We substantiate this mechanism by computational modeling, which recovers the unique shapes observed for the most ubiquitous caveolar clusters. We support the agreement between the calculated and observed configurations by electron tomography of caveolar clusters. The model predicts, quantitatively, the experimentally assessable dependences of caveolar clustering on membrane tension and on the degree of polymerization of the caveolin-cavin coats. We propose that the role played by large caveolar clusters in cells is to substantially reinforce the mechano-protective function of caveolae.

Abstract - The Mentor's Lecture 3

Biohybrid Implant Development – The role of mechanobiological stimuli

Stefan Jockenhövel et al.

NRW-Schwerpunktprofessur Biohybrid & Medical Textiles (BioTex)
Applied Biomedical Engineering – Helmholtz Institute Aachen
Forkenbeckstr. 55; 52074 Aachen, Germany

Abstract:
Regenerative Medicine has promised to overcome the limitations of conventional implants with the potential to remodel, to self-repair and specifically for the pediatric applications to grow with the child. In the past two decades, many successful pre-clinical trials have demonstrated the potential of tissue-engineered implants, but the number of translated products to the clinic are very limited. This is due to the high complexity of the production process and the need to control the complex adaptive behavior of the patient-individualized cell source in the process.

While the classical tissue-engineered implant has primarily focused on a complete autologous solution, the biohybrid approach is looking for a balance combination of technical and biological components with regard to a high (re)producibility by the technical component and an optimal hemo/biocompatibility by the biological component.

In principle the fate of biohybrid implants is defined by three major aspects (i) the cell, (ii) the biomaterials and (iii) the stimuli, which influencing the differentiation and "productivity" of the cells for generating a physiological functional tissue. Stimuli can be (bio)chemically but more over the research has demonstrated the essential need of mechanobiological stimulation during the tissue development.

The presentation will give an inview in the development of biohybrid implants and influence of mechanobiological stimuli for the successful production of autologous implants.

Abstracts

Abstract Number = Poster Number

Abstract 1

POLARIZED EPITHELIAL CELL MIGRATION DEPENDS ON REAR END STRESS FIBER TENSION

Galiya Sakaeva[1] , Dmytro Kotsur[2] , Kritika Sahni[1] , Georg Dreissen[1] , Julian Mattes[3] , Bernd Hoffmann[1] , Rudolf Merkel[1]

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[3] **MATTES Medical Imaging GmbH, Hagenberg, Austria**

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KEYWORDS: epithelial cell migration, actin, laser ablation, stress fiber, keratinocyte, cell polarity

Actin stress fibers (SFs) play a central role in cell adhesion and migration. Recently laser ablation has become a popular tool to understand mechanical properties of actin SFs and to measure forces generated by a single or multiple actin SFs in non-locomoting or patterned cells, but still less is known about the role of individual actin SFs in migrating cells. In this study laser nanosurgery combined with live-cell microscopy was applied to understand several aspects of actin SFs contribution cytoskeletal organization. First, the input of the actin ventral SF at the rear end of the polarized cell to cell polarity and migration in human keratinocytes (NHEK) was analyzed. The second part was devoted to investigation of a contribution of the SFs of different localizations in cell, namely, SFs in lamellae, in support of cell polarity and locomotion. And finally, mutant murine keratinless keratinocytes were used as a convenient cell model to better understand participation of intermediate keratin filaments in cell polarity.

We showed that severing of the actomyosin rear end SF results in subsequent disassembly of the composing it proteins from the ablated ends: myosin II, α-actinin and zyxin which reflect loss of tension in the ends of the cut SF. The loss of tension was followed by generation of a new actin fiber at the sire of ablation. Formation of the new actin fiber included incorporation of these molecules but was insufficient to maintain the rear end, and at the site of ablation a cell membrane started protruding in a mode of lamellipodium, finally leading to global cell shape reorganization, pancake-like cell shape and temporal stop of migration.

Laser destruction of the ventral SFs in lamellae demonstrated the importance of a structural integrity of this SFs for a preserved cell polarity and migration. The cells demonstrated the following behavior upon the laser ablation: formation of the membrane protrusion at the rear end, loss of polarity, acquisition of a circle-like shape accompanied by the vanishing of ventral SFs, generation of transverse arcs and dorsal SFs and halt of migration.

Keratin filaments in its turn were shown not to possess an inevitable role in cell polarity and migration – in both types of the local laser ablation experiments, laser cuts of the rear end SFs and ablation through all the SFs in lamellae, the keratiness keratinocytes demonstrated cognate reorganization of the cell shape as in the experiments with the nHEKs expressing keratins, briefly, formation of the membrane protrusion at the rear end and loss of polarization.

Thus, laser nanosurgery proved to be a useful tool in understanding of the local importance of the cytoskeletal structures and, in particular, that myosin-derived tensile SF at the rear end of the polarized migrating cells as well as the structurally integral SFs in lamellae are of great importance for cell polarity and migration.

Abstract 2

Focal adhesion - hemidesmosome crosstalk in migrating keratinocytes

Anne Pora, Reinhard Windoffer, Rudolf E. Leube

Institute of Molecular and Cellular Anatomy, RWTH Aachen University, 52074 Aachen

Keratin-anchoring hemidesmosomal cell-matrix anchoring sites contribute to the mechanical integrity of epithelia. Their role in epithelial cell migration, however, remains unclear. We find that hemidesmosomes cluster in migrating human primary keratinocytes as arrays consisting of multiple chevrons that are flanked by actin-associated focal adhesions. The arrays extend from the cell rear to the cell front. New hemidesmosomal chevrons form subsequent to focal adhesion assembly at the cell's leading front whereas chevrons and associated focal adhesions disassemble at the cell rear. The bulk of the hemidesmosome-focal adhesion composite, however, remains in place during cell translocation. Similar hemidesmosome-focal adhesion patterns emerge during substrate adhesion and cell spreading. We further find that hemidesmosomes and focal adhesions affect each other's distribution. Our results provide evidence that both junctions are separate but linked entities that treadmill coordinately to support efficient directed cell migration. They further suggest that both junctions cooperate to coordinate the dynamic interplay between the keratin and actin cytoskeleton.

Abstract 3

EGF triggers Rho excitability in migrating keratinocytes

Patwardhan R., Koch, J., Graessl, M., Kamps D., Calderon, A., Schulze, N., Jungkurth, J.K., Dehmelt, L., Nalbant, P.

Abstract

Growth factor stimulation is known to trigger migration in several cell systems, including primary human keratinocytes. Interestingly, in epidermal growth factor (EGF) stimulated keratinocytes, migration is preceded by a short period of protrusion and contraction in the cell periphery. However, the mechanism that controls this dynamic cell behaviour and its relevance for cell migration are poorly understood. We recently revealed a signal network that generates local pulses and propagating waves of cell contraction (Graessl et al., 2017). These dynamic patterns emerge from a combination of positive and negative feedback regulation of Rho activity. Rho rapidly amplifies its local activity by recruiting Rho-GEFs to the plasma membrane, and it inhibits its activity on a slower timescale by recruiting acto-myosin and associated RhoGAPs. Dynamic Rho activity patterns are stimulated by increasing the concentration of the Lbc-type GEFs GEF-H1 or LARG, but not by the non Lbc-type GEF Ect2. Here, we showed that GEF-H1 stimulated Rho excitability is also a feature of primary human Keratinocytes, and that acute EGF stimulation can trigger a single major Rho activity peak in the cell periphery that was followed by local cell contraction. This suggests that growth factor signalling can trigger Rho excitability during the initiation of cell migration. Conceivably, Rho excitability could also play a role during later stages of cell migration, in which cells polarize to generate a leading front and trailing back. However, during persistent migration of Keratinocytes following EGF stimulation, only low-amplitude Rho activity pulses were observed throughout the cell attachment area with no obvious preference along the cell polarization axis. In contrast, we observed high level activity of the related Rho family member Cdc42 at the trailing edge of polarized, migrating Keratinocytes. This was surprising, as Cdc42 is not thought to play important roles in the trailing edge of migrating cells, but instead is a well-known regulator of leading edge dynamics (Nalbant et. al., 2004; Machacek et. al. 2009; Yang et. al, 2016). To verify this surprising observation, we performed knock-down experiments and show that the high Cdc42 sensor signal at the trailing edge indeed is derived from endogenous Cdc42 activity. Furthermore, Cdc42 knockdown led to a reduction of migration speed and Euclidean migration distance, suggesting that this unusual Cdc42 activity pattern might play an important role in migration efficiency and persistence of migration directionality. Rho activity pulses might play an additional role to modulate cell contraction dynamics on a smaller, local scale that is independent of the general cell polarization axis.

Publications:

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Abstract 4

Victor Juma

University of Sussex, School of Mathematical and Physical Sciences, Mathematics

Parameter identification for Rho-myosin model

Abstract 5

Collective cell migration: intercellular forces coordination by integrin α5β1

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Collective cell migration is a fundamental biological process characterizing embryogenesis, cancer progression and wound healing. At the singular cell level the mechanism regulating the migration process is the coordination between cell-cell adhesion structures and cell-extracellular (ECM) adhesion. Many studies from different groups, including our, have already elucidated the role of junctional components and their regulation during migration, but little is still known about the regulation of cell-ECM adhesion and how these two adhesion sites "communicate" to coordinate collective migration.

The aim of this work is to understand the role of the mechano-responsive α5β1 integrin receptor in the mechanobiology of epithelial cell migration.

We developed an experimental approach using polyacrylamide gels (PAA) nanopatterned with highly specific α5β1 integrin peptidomimetics at discrete spacing, such as 35, 50 and 70nm, in order to control receptor densities. A monolayer of keratinocytes (HaCaT) is seeded on the coated hydrogels in a confined area, and then allowed to migrate towards the available space, mimicking a wound healing process.

The use of hydrogels as surface for cell migration not only better mimics the physiological stiffness of the skin dermis but give us the possibility to employ mechanobiology techniques that allow both qualitative and quantitative evaluation of adhesive forces at the ECM (traction force microscopy) and at cell-cell junctions (monolayer stress microscopy).

Here we present data suggesting an optimal integrin α5β1 density, which is necessary in order to control cell-cell force orientation and therefore an optimal collective coordination during migration. On-going work elucidates the pivotal intracellular players that control transmission of cell-ECM traction forces to cell-cell stresses.

Abstract 6

The basement membrane: A mechanical cell invasion barrier in breast cancer.

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Among women, breast cancer is the most frequently diagnosed cancer and the leading cause of cancer deaths worldwide. The processes steering breast cancer invasion are still less understood. It has been hypothesized that basement membrane disruption and cell invasion could be mechanically triggered by loss of tissue homeostasis and force imbalances. Therefore, we used the 3D MCF10A acini cell culture model to investigate to what extent basement membrane integrity counteracts cell invasion. Tumor associated extracellular matrix (ECM) stiffening, aberrant growth factor signaling, and increasing cellular forces were analyzed on their ability to induce basement membrane disruption and cell invasion.

By applying living acini to a newly designed basement membrane invasion assay, the onset and the overall incidence of invasion were determined in dependency ECM stiffening, aberrant growth factor signaling and gradually decreased basement membrane integrity - in order to resemble progressive breast tumor tissue conditions.

We could demonstrated that substrate stiffening triggered invasion onset and increased the overall invasion incidence. Cell-basement membrane transmigration was clearly fostered by an additional tumor-related epidermal growth factor (EGF) stimulus. Interestingly, the sensitivity of BM-covered breast acini to ECM stiffness was clearly overwritten after growth factor stimulation. In contrast, the importance of basement membrane stability could be confirmed as paramount mechanical invasion barrier under all experimental conditions. Traction force microscopy (TFM) analyses demonstrated progressively increasing cellular forces during cell-mediated basement membrane breakdown and outgrowth. Tumor-like ECM stiffness considerably contributed to generation of higher force amplitudes. By using elastic resonator interference stress microscopy (ERISM), spatially localized vertical substrate deformations were detected in acini with a still intact basement membrane shell at very early invasion stages. In line with this, immunostainings revealed the presence of actin-rich, finger-shaped basement membrane spanning protrusions. This strengthens the hypothesis of acinar cell protrusions that could function as ECM stiffness sensing units being involved in basement membrane invasion.

Overall, we demonstrated that breast cancer cell invasion is activated by ECM-stiffening and aberrant EGF signaling. Our findings clearly highlight the crucial role of basement membrane integrity as mechanical gatekeeper of cell invasion. Detailed characterization of acinar cell protrusions as potential mechanosensors for cell invasion will be in scope of upcoming studies.

Abstract 7

In vitro models for studying endometrial receptivity - characterization of human biopsies in a new test system

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Objectives: A basic requirement for human embryo invasion which comprises penetration of endometrial epithelial cells (EECs) by trophoblast cells is the appropriate preparation of the endometrium. Cyclic differentiation of endometrial cells, which is controlled by ovarian steroid hormones, leads to a short receptive period called window of implantation (WOI). As we could show previously, the WOI is characterized among other histological and biochemical changes by an altered distribution of adhering junctions in EECs which indicates a change in EEC polarity during the WOI.

Methods: To elucidate basic mechanisms of early human implantation we established a new 3D cell culture confrontation system. In this cell culture system we studied trophoblast-endometrial interaction by confronting gland-like spheroids of different endometrial adenocarcinoma cell lines with trophoblast cells. We further focused on the study of primary endometrial cells from scratch biopsies of women undergoing assisted reproductive technology (ART).

Results: Using confocal microscopy we could show that EEC cell line spheroids with a junction distribution similar to EECs during the WOI in vivo were more strongly invaded by the extravillous trophoblast cell line AC-1M88 than highly polarized EEC cell lines. First results on primary EEC cultures could be obtained showing close trophoblast-endometrial interactions. By use of light sheet microscopy we achieved a particularly high resolution of the invasive processes in real-time. Further studies are in progress to analyze the influence of 17beta-estradiol and progesterone on EEC receptivity and the invasiveness of trophoblast cells in the 3D confrontation culture system.

Conclusion: By applying a newly established 3D trophoblast invasion assay on tissue samples from patients undergoing ART, we expect new insights into basic mechanisms of human implantation. Data obtained from this new confrontation approach may help to find new therapeutic strategies in ART treatment.

Abstract 8

Regulation of fractalkine-induced monocytic cell recruitment by endothelial shear stress

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Flow conditions critically regulate endothelial cell functions in the vasculature. Reduced shear stress resulting from disturbed blood flow can drive the development of vascular inflammatory lesions. On endothelial cells the transmembrane chemokine CX3CL1/fractalkine promotes vascular inflammation by functioning as a surface expressed adhesion molecule and by becoming released as soluble chemoattractant for monocytic cells expressing the receptor CX3CR1. Here we report that endothelial cells from human artery, vein or microvasculature constitutively express CX3CL1 when cultured under static conditions. Stimulation with TNFα under static or very low shear stress conditions strongly upregulates CX3CL1 expression. By contrast, CX3CL1 induction is profoundly reduced when cells are exposed to higher shear stress. When endothelial cells were grown and subsequently stimulated with TNFα under low shear stress, strong adhesion of monocytic THP-1 cells to endothelial cells was observed. This adhesion was in part mediated by transmembrane CX3CL1 as demonstrated with a neutralizing antibody. By contrast, no CX3CL1 dependent adhesion to stimulated endothelium was observed at high shear stress. Genetic disruption of the receptor signaling in leukocytes via the coupled G protein did not prevent adhesion to endothelial cells suggesting that the physical interaction between the receptor and its ligand is sufficient for this event. By contrast, receptor signaling was clearly required for the subsequent transmigration of the endothelial cell layer. Thus, low shear stress typically seen at atherosclerosis prone regions promotes the induction of endothelial cell expressed CX3CL1 which promotes monocytic cell adhesion by physical interaction with CX3CR1 and subsequent leukocyte recruitment by receptor signaling.

Abstract 9

Novel strategy to determine cancer cell heterogeneity *in vitro:* assaying the relation force/speed/morphology with micro-featured scaffolds

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Biomaterials mimicking the mechano and topographic features found in neoplastic milieus have arisen as an extremely interesting option for testing cell pathogenicity, and for assaying cancer heterogeneity.

Our research is currently focused on performing new featured hydrogels to dynamically characterize the metastatic potential of breast cancer cells *in vitro*. Our study is based on the simultaneous determination of cell morphology, traction forces and migration speed of metastatic and healthy single-cells. The combination of such parameters may allow us to determine the existence of multiple phenotypes (*i.e.* malignant heterogeneity) within an entire population.

To achieve this aim, soft polyacrylamide hydrogels, having an elasticity of 10 kPa (measured as Young's moduli) were patterned with thin migration lines (4 µm width and 100 µm length), made of collagen type I and fibronectin. Such features were localized in a miniaturized scaffolds (22X22 mm), which was designed to mimic mechanical and topographical cues found at the ECM of tumoral niches.

Initially, experiments were performed with wild-type breast cells (MCF10A; non-metastatic). Control cells exhibited the existence of two sub-populations within the original phenotype, differentiated in terms of length, speed, and exertion of traction forces. To compare the behavior of these cells, experiments were also performed with the metastatic phenotypes MCF10A-ErbB2 or MCF10A cultured in presence of TGF-β. Our results are showing that both MCF10A-ErbB2 cells and MCF10A cultured in presence of TGF-β shifted their distribution pattern within the entire population, being characterized by the apparition of a dominant phenotype, defined by their small and round morphology, fast migration, and exertion of low mechanical energy (weaker traction forces).

According to our preliminary data analysis, it is possible to indicate that our result may represent the first attempt to study *in vitro* cell heterogeneity using soft featured scaffolds, offering a new technological alternative to traditionally used microfluidic devices.

Furthermore, our experimental findings are showing that this information cannot be assessed by averaging experimental results obtained from an entire population (*i.e.* considering this as a homogeneous and uniform group of cells), rather than extracting tendencies from analyses performed at single-cell scale, pondering the potential existence of cell sub-types within a multicellular experiment.

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Abstract 10

β1 subunit stabilises sodium channel Nav1.7 against mechanical stress

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Voltage-gated sodium channels (Navs) are key players in neuronal excitability and pain signaling. Precise gating of these channels is crucial as even small functional alterations can lead to pathological phenotypes such as pain or heart failure. Mechanical stress has been shown to affect sodium channel gating. This suggests that components which stabilize the channel are necessary to ensure precise channel gating in living organisms.

We used whole cell patch clamp of Nav1.7 heterologously expressed in HEK cells in presence and absence of the β1 subunit to study the impact of the β1 subunit on mechanical susceptibility of Nav1.7. All groups were measured in the presence and absence of mechanical stress applied by the flow of extracellular solution on the patched cell via a gravity-driven perfusion system. We show that mechanical shear stress shifts voltage dependence of activation (ΔV_{half}= 10.5 mV, p= 0.0004), and of fast inactivation ((ΔV_{half}= 6.3 mV, p=0.04) to more depolarized potentials. Co-expression of the β1 subunit, however, protects both voltage dependence of activation and fast inactivation of Nav1.7 against mechanical shear stress.

To study the underlying mechanisms enabling the β1 subunit to mechanically stabilize Nav1.7, we used molecular dynamics simulations (MD), homology modelling and site-directed mutagenesis. MD simulations were carried out using GROMACS 2016 using the β1 structure published by Yan et al. 2017. Simulations of WT β1 and the C43A mutant revealed an intramolecular disulfide bond of β1 (Cys21-Cys43) to be crucial for the correct forming of the binding site within the subunit. Using a homology model of Nav1.7 based on the Nav1.4β1 Complex (Yan et al. 2017), we show that β1 binds to segment 6 of domain IV of Nav1.7. To investigate the functional impact of the mutation we generated a C43A β1 subunit by site directed mutagenesis. Whole cell patch clamp experiments with the C43A β1 subunit and Nav1.7 show that the mutant prevents mechanical modulation of voltage dependence of activation, but not of fast inactivation.

Our data reveal a novel feature of β subunit-mediated ion channel modification: the β1 subunit is able to influence the mechanical susceptibility of Nav1.7. Furthermore our patch data emphasize the unique role of segment 6 of domain IV for sodium channel fast inactivation and confirm previous reports that fast inactivation can be modified by interfering with the extracellular end of segment 6 of domain IV. Our study suggests that physiological gating of Nav1.7 may be protected against mechanical stress in a living organism by assembly with the β1 subunit.

Literature:

Yan Z, Zhou Q, Wang L, Wu J, Zhao Y, Huang G, Peng W, Shen H, Lei J & Yan N (2017). Structure of the Nav1.4-β1 Complex from Electric Eel. Cell 170, 470–482.e11.

Abstract 11

Advanced continuum modeling of lipid bilayer membranes

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Cellular membranes composed of lipid bilayers are characterized by complex mechanical behavior that is very challenging to describe accurately at continuum length scales. Cellular membranes undergo large shape changes, they exhibit non-trivial surface flows, they undergo phase transitions, and they interact with surrounding objects. Modeling those requires solving coupled partial differential equations on evolving surfaces.
This presentation gives an overview of a new, three-dimensional modeling framework that has been developed recently in order to describe and simulate the mechanical behavior of cellular membranes. It is based on advanced modeling techniques in mathematics, physics and computational analysis. Its mathematical description is entirely formulated in the general theory of differential geometry, since this is capable of describing arbitrary surface flows and shape changes. Its physical description is based on a rigorous thermodynamical treatment, since it provides coupling relations for the various physical fields. Its computational description is based on so-called isogeometric surface finite elements, since they are capable of representing surface flow, shape and constitution to high accuracy. The capabilities of the new framework are illustrated by several challenging numerical examples.

Abstract 12

A COUPLED BULK-SURFACE MODEL FOR CELL POLARISATION

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Cell polarisation is the result of intricate networks of biochemical reactions. Recent work on balancing biological complexity with mathematical tractability resulted in the proposal and formulation of a famous minimal model for cell polarisation, known as the wave pinning model. In this study, we present a three-dimensional generalisation of this mathematical framework through the maturing theory of coupled bulk-surface semilinear partial differential equations in which protein compartmentalisation becomes natural. We show how a local perturbation over the surface can trigger propagating reactions, eventually stopped in a stable profile by the interplay with the bulk component. The bulk-surface finite element method is used to generate numerical simulations over simple and complex geometries, showing pattern formation due to propagation and pinning dynamics. The generality of our mathematical and computational framework allows to study more complex biochemical reactions and biomechanical properties associated with cell polarisation in multi-dimensions.

Abstract 13

Dynamic interaction of cytoskeletal filaments in migrating keratinocytes

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Cytoskeleton dynamics are highly regulated and important for proper cell migration. The most abundant cytoskeletal components in epithelial cells are the keratin intermediate filaments. They have to interact with the other cytoskeletal components, microtubules and actin filaments, and probably with their motor proteins. However, the nature of these interactions during epithelial cell migration are mostly unknown. By combining cytoskeletal targeted drug treatments in primary human keratinocytes with several imaging techniques and micropatterning, we aim to dissect the interdependence of the three major cytoskeleton components of epithelial cells during cell migration. Using image analysis tools, keratin dynamics can be compared to movement of the other cytoskeleton components. This has led to the observation that the actin-myosin system moves from the plasma membrane toward the cell interior together with newly-assembled keratin particles until it encounters the keratin filamentous network, whereupon its movement slows down and separates from the keratin system. Thus, a clear border between the actin and keratin cytoskeleton is delineated in the migrating keratinocytes. The keratin and microtubule networks do not co-localize in large parts of the cytoplasm. Nonetheless, newly forming keratin filaments seem to use microtubules as a template. Annihilation of both the microtubules and the actin filaments leads to a stepwise relaxation of the keratin network, confirming our observations for the dynamic interactions between keratin and these two systems separately.

Abstract 14

Automatic segmentation and tracking of keratin filaments in living cells

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Keratin filaments form a highly branched dynamic network, which impacts the mechanical properties of epithelial cells and which protects them against mechanical stress and damage. This network has highly variable properties differing from one tissue (cell type) to another. The investigation of the network properties requires a deep and accurate analysis of a huge amount of image data, which would be extremely time-consuming and difficult to accomplish manually.

Therefore, we present an image analysis tool, which allows to segment and track the trajectory of individual keratin filaments within their network. The segmentation procedure is based on the Voronoi medial axis, which allows to obtain initial position of the filaments with sub-pixel accuracy. The tracking procedure employs an active contour model (snake), which we extend by an additional term for an accurate snake's endpoints tracking. The proposed algorithm was evaluated on 300 ground truth trajectories using different error measures and compared with two state-of-the-art methods. We also investigated the problem of automatic hyper-parameter tuning for the snake algorithm, especially heuristic parameter choice rules with respect to the gradient vector flow problem.

Abstract 15

A computational framework for simulating the dynamics of keratin filament networks

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[3] MATTES Medical Imaging GmbH (MATTES), Hagenberg, Austria

In this work, we propose a biomechanical computational framework, which allows us to perform numerical simulations in order to mimic the behaviour of keratin filament networks as observed in microscopy data. The developed automated computational methods allow us to: (1) generate, by a randomized process, a filament network topology with predefined properties; (2) simulate the motile behaviour of the filament network; (3) synthesize image data based on the produced simulations. The synthetic image sequences can then be used to evaluate filament extraction and tracking algorithms as the underlying ground truth is available.

The core of our simulation module consists of a spring-mass-damper discrete mechanical system, which allows simulating elastic behaviour of the filaments. Additional stochastic external forces are also incorporated, which allow exhibiting the effect of Brownian motion and the influence of the actin network resulting in an inward-directed filament flow.

Based on the produced simulations we are able to synthesise artificial image data mimicking the behaviour of the filament network. Artificial images can be exposed to additional microscopy-related imaging artefacts such as Poisson/Gaussian noise, convolution with a predefined point spread function.

Abstract 16

A Bayesian approach to parameter identification: keratin dynamics and traction force microscopy

Eduard Campillo-Funollet

September 19, 2018

We present a Bayesian approach to parameter identification, emphasising the advantages of the method. Although the approach has a higher computational cost than the alternative optimisation approach, it allows us to incorporate in detail the current knowledge about the problem.

To illustrate the applicability of the method, we present results on two problems in mathematical biology. First, we identify the space-dependent reaction rates in a model for the dynamics of the keratin network. Second, we compute the force at the focal adhesions using traction force microscopy data.

Abstract 17

Active composite agents: a minimal model for motile cells

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The cytoskeleton is a highly dynamic three-dimensional network of polar filamentous proteins and molecular motors. It provides structural stability for biological cells and generates and transmits mechanical forces. For example, in mesenchymal cell motility actin filaments polymerize at their plus ends, which exerts pushing forces on the cell membrane. Here, we present a generic model of flexocytes, where self-propelled filaments attached to deformable rings form a mechanosensitive self-propelled agent. We interpret shapes and motion predicted by our model in the context of motile cells. To further test our model, we probe the effect of the substrate patterning on the mechanical response of the active flexocytes.

The flexocytes reproduce experimentally observed motility patterns of biological cells and assume circula, keratocyte-like and neutrophil-like shapes. Interestingly, explicit pulling forces only are sufficient to reproduce this cell-like behavior. In particular, we study the interaction of flexocytes with obstacles and at friction interfaces and find parallels to the behavior of biological cells. Our model may thus serve as a filament-based, minimal model for cell motility.

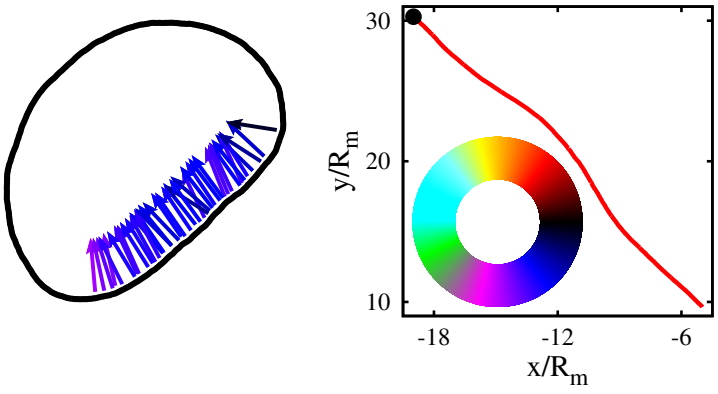

Fig. 1: *Self-propelled flexocytes with a keratocyte-like shape. The left plot shows a flexocytes with explicit pulling forces only. The right plot shows the trajectory of the center of mass of the flexocyte. The black point represents the position for the last frame. The color wheel indicates the rod orientation.*

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Abstract 18

Precise microfluidic clustering of multiple cell types for quantifying paracrine effects in cancer cells

Nikos Fatsis-Kavalopoulos[a,b] ,Beichen Xie[a] ,Carl Andrén Lundahl[a] ,Rodrigo Hernández Vera[a] ,Olof Idevall-Hagren[a] ,Paul O'Callaghan[a] and Johan Kreuger[a]

The study of the interactions between cells within a group is imperative in understanding the complex dynamics of collective cell behaviour. Cell interaction through paracrine ATP signalling has been shown to be a key contributor in directed cell migration and chemoattraction. ATP itself has been a point of study as a stimulant that leads to cytoskeletal and cell polarity changes. In this paper we present a novel way, in the form a microfluidic device, to form precise paracrine response models to study the collective behaviour of cells. This microfluidic device, called the Cell Assembly Generator (CAGE), enables the formation of cell groups of a predetermined cell composition. First cells are isolated from entire cell populations, using hydrodynamic single cell trapping, then those cells are clustered in a specifically designed cluster chamber. By repeating the protocol of trapping and clustering multiple times, multicellular clusters are formed one cell at a time. With the way the CAGE is designed, both the exact number and the type of cells put into a cluster can be determined and multiple identical clusters can be formed simultaneously in every device. Using CAGE Multiple study clusters were generated to model in a precise way intracellular calcium modulation in response to paracrine ATP stimulation.

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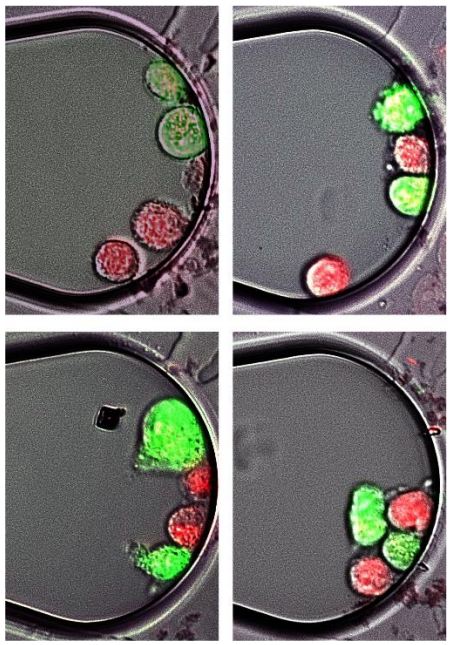

Abstract 19

TissueFAXS Cytometry – A Tool for Precision Medicine: Automated Analysis of Single Cells and the Cellular Microenvironment

Rupert Ecker
Chief Executive Officer, PhD
TissueGnostics GmbH
Austria

Abstract

Determining the in-situ immune status of diseased organs or quantify coexpression of molecules on the single-cell level has mostly been subject to visual estimation, or – at best – to manual counting for decades. Hence, experts usually had the choice of the "least of evils" between *guessing* and *endless (manual) counting*. In tumor immunology, infiltrating inflammatory cells need to be phenotypically characterized on a quantitative basis. To better understand the function of inflammatory cells in tumor development, type and number of inflammatory cells and their proximity to glandular/tumor structures have to be analyzed in-situ and correlated with disease state. Using TissueFAXS™ Cytometry the time-consuming and error-prone human evaluation of stained histological sections can be approached with an observer-independent and reproducible technology platform, offering a high degree of automation, paired with user interaction at relevant points of the analytical workflow. This platform can be applied as a means of tissue cytometry for both immunofluorescence and immunohistochemistry and thus constitutes the microscopic equivalent to flow cytometry (FACS).

Likewise FACS, TissueFAXS™ can quantify any type of molecular marker in any type of cell – but in tissue context or in adherent cell culture monolayers without the need to solubilise the cells (i.e. TissueFAXS permits analyses *in-situ*)!

The TissueFAXS Cytometry platform can be used in clinical multi-center studies to determine the immune response to certain drugs *in-situ*, measure proliferation, apoptosis, cytokine expression, signalling molecules, and others. It can do end-point assays as well as live-cell imaging and time-kinetic experiments. But TissueFAXS Cytometry also promotes tissue cytometry to a new level of quality, where complex cellular interactions can be addressed on the single-cell level but still in histological context.

Biography

Dr. Rupert Ecker has been co-founder of the TissueGnostics group in Austria, Romania, and USA. He is Chief Executive Officer in TissueGnostics-Austria and TissueGnostics-Romania, Vice President of TissueGnostics-USA, as well as Area Manager of TissueGnostics China Division.
Before founding TissueGnostics he was a research scientist at the Competence Centre for Bio Molecular Therapeutics in Vienna, a joint venture between the University of Vienna and the Novartis Research Centre.
As a co-inventor of the TissueFAXS technology Rupert Ecker has always been significantly involved in research and (product) development from system design to clinical testing and has successfully headed several joint R&D projects with academic partner institutions in the fields of advanced computer vision, cancer research, stem cell biology, and personalized medicine.
Rupert Ecker graduated in Cell Biology from the University of Vienna. He has more than 20 years of experience in microscopy and image analysis. In addition, he has been trained in software development.

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Abstract 20

Keratinocytes migrate individually within a collectively migrating sheet during wound closer *in vivo.*

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Abstract:

Epidermal wound healing is a crucial process to re-establish the protective barrier function of the skin. However, how epidermal keratinocytes dynamically migrate in order to close the wound has not been analysed *in vivo,* yet. Therefore, we established an *in vivo* scratch wound system within the back skin of a mouse to follow individual keratinocytes in the epidermal sheet during the course of wound healing using intravital microscopy.
We discovered that single keratinocytes migrate independently within a collectively migrating epidermal sheet. Moreover, we show that keratinocytes change their direct neighbouring cells, indicating that they remodel their cell-cell-junctions during migration. Additionally, these dynamics of single cell migration within a migrating sheet enable keratinocytes to overcome obstacles such as hair follicles.
Taken together using a newly developed *in vivo* system we can uncover the unexpected migratory behaviour of individual keratinocytes within the collectively migrating sheet.

Abstract 21

Cell force-mediated breast cancer invasion is attenuated by basement membrane integrity

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Among women, breast cancer is the most frequently diagnosed cancer and the leading cause of cancer deaths worldwide[1]. Despite extensive research, the processes involved in invasion of malignant breast cancers are still not fully recognized. An improved understanding of these processes would facilitate effective therapy and hinder a malignant tumor from invading the adjacent tissue.
In vivo, the human breast gland tissue consists of polarized luminal cells which are surrounded by a basement membrane (BM) which forms a physical barrier separating the breast gland from adjacent extracellular matrix (ECM). The main structure lending component of the BM is collagen IV, which is endogenously produced by epithelial cells. During embryogenesis and tissue differentiation BM scaffold also develops in terms of thickness and stability[2-3]. In a recent study we characterized this time dependent BM formation *in vitro* by using MCF10A breast gland acini[4].
Here we analyzed the reciprocal interplay between tumor-associated ECM-stiffening and developmental state of the BM. We hypothesize that BM disruption and transmigration can be triggered by extracellular durotactic cues and investigated to what extent BM integrity suppresses cell invasion. Using life cell imaging, mean invasion onset time points and overall incidences of cell-BM transmigration were determined in dependency of both, normal breast- and tumor-like ECM stiffness. Moreover, we determined whether, and to what extent, BM disruption is accompanied by altered cell force generation and quantitatively characterized the local invasion process in detail by traction force microscopy (TFM). Cellular transmigration through the BM was additionally induced or suppressed by invasion relevant biochemical factors: epidermal growth factor and collagenase IV to trigger invasion, matrix metalloproteinases (MMPs) inhibitor marimastat, and phosphoinositide 3-kinase (PI3K) inhibitor wortmannin as suppressors.
Interestingly we found that BM integrity has the largest impact on invasion onset and incidence, whereas ECM stiffness showed an ancillary effect. In line with this, MMP inhibition resulted in a conspicuous invasion decrease, but importantly, not in a complete abolishment. This indicates that there might be another mechanism for transmigration, despite enzymatic weakening of the BM. In contrast, degradation of the BM by collagenase IV showed that the BM was no longer able to repress cellular invasion, thus emphasizing the role of the BM as invasion barrier. Interestingly, TFM analyses revealed progressively increasing cell forces during cell-mediated BM-breakdown and outgrowth. Additionally, the tumor-like ECM stiffness considerably contributes to generation of higher forces. We finally aimed to decipher the molecular key player by which invasion might be activated, and could demonstrate that PI3K seems to be a crucial factor, as its short inhibition let to a significant decrease of invasion incidence and retarded invasion onset.
Our results demonstrate that the key mechanism of cancer cell invasion is not only a proteolytic, but also cell force-mediated BM disruption and durotaxis-driven BM transmigration mechanism. These findings highlight the crucial role of BM-integrity as a mechanical barrier against breast cancer cell invasion.

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Abstract 22

Assessment of biomechanical properties of cancer cells pre-cultured on 2D and within 3D tumor-like scaffolds

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Keywords: Artificial Tumors; 3D cell culture; Cell encapsulation; Cell traction force; Artificial neoplastic niches.

Abstract

Accurate *in vitro* biophysical modeling of the neoplastic niche may hold the key to enhanced understanding of the mechanism of cancer progression. One of the ongoing challenges in malignancy is determining the influence of three dimensional (3D) confinement on the morpho-biomechanical activity of neoplastic cells. To address this issue, we characterized a pathogenic cell line MCF7 in terms of morphology and traction forces, after culturing the cells on 2D or immobilizing them within 3D microenvironments (*i.e.* tumor-like microcapsules).

For 2D culture, the MCF7 cells were grown in cell culture flasks. To study the effects of 3D microenvironments however, MCF7 cells were immobilized within 600 µm alginate/gelatin spherical tumor-like microcapsules. These were designed to mimic the environment of an *in vivo* neoplastic niche, characterized by the restricted oxygen supply, restricted biodegradability, as well as enhanced mechanical stress from the surrounding stiffness (20.0 kPa). It is relevant to note that alginate/gelatin provided a partially biodegradable pathway for the cells to escape the entrapment (*i.e.* cell release from the tumor-like microcapsules was observed 7 days after the immobilization). After pre-culturing MCF7 cells on 2D and within 3D milieus, delivered cells were deposited onto two substrates: sterile glass or polyacrylamide hydrogel (PAA). The area of the cells was determined on both substrates by Image J. In addition, cells were seeded on PAA films (8.0 kPa) to measure traction forces.

Our results indicate clear differences in cell morphology and mechanical activity, depending on the nature of the scaffolds used to pre-cultivate the neoplastic cells. These findings suggest that the strategy of performing *in vitro* experiments may benefit from the use of 3D milieus instead of just bare 2D surfaces.

Abstract 23

Automated tracking of keratin granules reveals effect of non-muscle myosin II on granule motility

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The characteristics of the keratin intermediate filament cycle of assembly and disassembly are well-established. They include the nucleation of nascent particles in the cell periphery, subsequent elongation, retrograde transport and fusion with the peripheral keratin network, and finally either integration into the perinuclear cage or disassembly into soluble subunits. The exact transport mechanisms of keratin are still not elucidated, but recent studies indicate that they might include motor protein-driven transport via actin or microtubules. In order to detect potential alterations of the keratin cycle by manipulating actin or microtubule motor proteins, automated large-scale analyses of keratin filaments are required. However, reliable systems for automated keratin tracking are still lacking.

Using live-cell imaging of MCF-7 cells expressing mutant keratins, which form mainly peripheral granules instead of filaments, we show that keratin granules display several features of the normal keratin cycle, thus providing an alternative readout system for keratin tracking. Automated granule tracking allowed the analysis of various readout parameters, such as granule speed, directionality, size alterations, fusion events and granule size prior to disassembly. Using the novel tools, we examined the crosstalk between keratin intermediate filaments and the actomyosin network by inhibiting non-muscle myosin II motor activity. Treatment of cells with blebbistatin significantly slowed down granule movement and altered other parameters of the granule cycle. The results provide proof-of-principle that the new image analysis tools are useful to quantify effects of pharmacological intervention on keratin dynamics.

Abstract 24

Migration component dynamics in epithelial cell motility

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Cell migration is a fundamental process in development and maintenance of multicellular organisms. A coordinated assembly and release of focal adhesions has been reported to be crucial for harmonious cellular movement. Actin and myosin IIA are pivotal components of the cytoskeletal network, that work in accord with each other to facilitate directed cellular movement. In order to characterize the continuous flow of molecules as well as protein exchange behavior in distinct structures during migration, we have used photoconvertable Dendra2 fusion proteins of actin, myosin IIA and vinculin in motility stimulated primary human epithelial keratinocytes. Following the photoconverted protein incorporation into non-converted structures, we have identified cytosolic diffusion of actin monomers and their subsequent exchange into different types of stress fibers, with very low exchange rates for transversal arcs but high incorporation rates for the rear-end stress fiber. In contrast, myosin IIA mobility was rarely measureable on the cytoplasmic level. Instead, myosin signal propagation took place exclusively along the existing actin stress fiber network. This myosin propagation speed was observed to correlate with the cell's mobility. For focal adhesions, our data confirm a faster exchange kinetics for nascent compared to mature complexes. Interestingly, the mature rear end focal adhesions were observed to switch their vinculin exchange kinetics to those of nascent ones shortly before disassembly. Furthermore, high resolution analysis indicate different distal-proximal exchange kinetics for disassembling focal adhesions at the cell rear with higher dynamics at their peripheral tips.

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Abstract 25

Advanced cell migration assay for studying chemotaxis in 2D or 3D environment

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We present a modified chemotaxis assay suitable for fast and simple analysis of chemotaxis of slow migrating cells, both in a 2D or 3D environment. The assay is based on µ-Slide Chemotaxis, a microfluidic chamber providing a long-term stable linear gradient, which is crucial for studying chemotaxis of slow moving cells, such as cancer cells or keratinocytes. Chemotactic response in the standard µ-Slide Chemotaxis is evaluated by videomicroscopy and consecutive analysis of migration tracks of individual cells. The real-time imaging of the cellular response to a chemical gradient provides an in-depth information on migratory behaviour; however, the data evaluation is a labour-intensive and time-consuming process. This presents a serious limitation for applications where increased experimental throughput is required, as it is typically the case of clinical examinations, chemoattractant screenings or studies of the chemotaxis-related signalling pathways based on subcellular perturbations. In order to simplify and fasten the assay, we equip the chemotaxis chamber with a hydrogel migration arena that restricts cell migration to a defined area of the chamber. The PEG-based, cell-repellent hydrogel is micro-structured inside of the chamber by photolitography. Restricting cell migration to the defined area renders it possible to detect chemotactic behaviour based on the cell distribution in respect to the end-point of the experiment. The end-point analysis approach, together with a simplified sample preparation procedure, enables processing of higher numbers of samples and cells in shorter time compared to the standard chemotaxis assay. The assay can be used to examine chemotaxis of cells migrating on a 2D surface, or embedded in a 3D extracellular matrix. As an example, the chemotactic effect of 10% fetal bovine serum on HT1080 fibrosarcoma cells in 3D collagen I gel was investigated. Further, we employed the assay to study the role of growth factors in the migration of human keratinocytes—the key cells involved in the process of wound healing; and identified the chemoattractant activity of epithelial growth factor (EGF) and transforming growth factor alpha (TGFα).

Abstract 26

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Assessment of biomechanical properties of cancer cells precultured on 2D and within 3D tumour-like scaffolds

Abstract 27

The interplay between Galectin-8 and fibronectin in the regulation of cell adhesion, spreading and morphogenesis

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Galectin-8 is a matricellular protein, a member of the galectin family, which is composed of tandem-repeats of two β-galactoside-specific carbohydrate binding subunits. Galectin-8 is a secreted protein that was shown to play an important role in angiogenesis, cell adhesion and migration and in the regulation of cell proliferation. Here, we explored the interplay between galectin-8 and fibronectin in the regulation of cell adhesion, spreading and morphogenesis. Live-cell, interference-reflection microscopy-based monitoring of HeLa, on substrates, coated with galectin-8. Our results indicated that the adhesion and spreading processes were β-galactoside-dependent and largely integrin independent, and were associated with an extensive formation of substrate-attached filopodia and lamellipodia. Using super-resolution microscopy, we found, in these cells, star-like actomyosin structures near the cell center, yet focal adhesions and stress fibers were hardly detected in them. In contrast, spreading on fibronectin was found to be highly integrin dependent and carbohydrate-independent process, and consisted of multiple protrusion-retracting cycles, resulting in a considerably reduced expansion, compared to cells spreading on galectin-8. Actomyosin organization on fibronectin was radically different from that, formed on galectin-8, and was characterized by multiple focal adhesions and acto-myosin-rich bundles, located at the cell periphery. Plating cells on surfaces coated with the two adhesion molecules, at different relative concentrations revealed the capacity of galectin-8 and fibronectin to drive cell protrusion and retraction, respectively, producing a combined dynamic and morphological response in the adhering cells, which was correlated to the relative concentrations of the two matrix molecules. These results shed new light on the physiological cell-matrix interaction, whereby cells sense, simultaneously adhesive signals from multiple components of the matrix, and have the capacity to integrate this complex information and develop a coherent response.

Abstract 28

Modeling cell shape changes by actin filaments

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Abstract

Cell shape and migration are controlled by dynamic actin cytoskeleton. In mathematical and physical modeling, representation of the interaction of actin filament with plasma membrane and the movement of plasma membrane remains a challenge [1]. Here we developed a simplified computational model of dynamic behaviour of the plasma membrane at the cell leading edge driven by polymerizing actin filaments. Our approach is based on Helfrich theory of membrane elasticity and Brownian dynamics. Taking advantage of the time scale separation of actin polymerization and plasma membrane equilibration, we minimize the membrane elastic energy. We developed a numerical optimization software based on quasi newton algorithm to get the minimum energy configuration of the cell leading edge. We report the computational result for different elastic parameters of the cell membrane.

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