Mechanobiology of Migrating Cells
From Basic Science to the Clinic

1st – 2nd April 2019

Venue
Department of Physics
Rua Larga, University of Coimbra
Dear colleagues,

Cell migration is pivotal in health and disease, namely during metastasis and in many processes during morphogenesis. However, it is a complex process where biochemistry, structural biology, physics and mechanics are intertwined. In cell migration, signalling pathways drive the concerted dynamism of cytoskeleton structure, leading to plasma membrane deformation and to precise localisation of mechanical forces, which (together with matrix remodelling) are capable of driving cell movement. Therefore, a better understanding of the mechanisms regulating cell migration can only be possible through a concerted effort by a multidisciplinary international collaboration, well in the spirit of EuroCellNet.

In this context, this meeting will try to provide a transfer of knowledge between top specialists on the different mechanisms of cell migration in health and disease. The meeting will bring together researchers of excellence which expertise includes: characterization of ECM properties, study of cell forces in 2D and 3D assays, study of cytoskeleton dynamics, development of state-of-the-art nanoscale solutions, and research the redox status of migrating cells. The present coupling of complementary experimental and modelling approaches will permit to develop a new collaboration at the Europe-wide scale with the aim of tackling the still existing challenges in elucidating cellular migration mechanisms.

We hope you enjoy the meeting.

Welcome to Coimbra.

Scientific Organising Committee
Ana Fernandes, Universidade Lusófona, Lisbon, Portugal
Nuno Saraiva, Universidade Lusófona, Lisbon, Portugal
Juan Carlos Rodríguez-Manzaneque, GENYO, Granada, Spain
Rui Travasso (Local Host), University of Coimbra, Portugal

Local Organizers

Sponsors
Monday, 1st April

09.45 - 10.00 WELCOME AND INTRODUCTION

Keynote Speaker

10.00 – 10.40 M. Ángela Nieto, Instituto de Neurociencias (CSIC-UMH), Alicante, Spain
“Epithelial plasticity in health and disease (the INs and OUTs of the EMT)”

ECM and Cell Migration

10.40 – 11.00 Florence Janody, University of Porto, Portugal
“Computational modelling and experimental approaches identify a role of ECM stiffening in Src-induced EMT”

11.00 - 11.20 Juan Carlos Rodríguez-Manzaneque, GENYO, Granada, Spain
“Relevance of ECM proteolytic remodelling for cell invasion and migration”

11.20 - 11.40 Rui Travasso, University of Coimbra, Portugal
“Mathematical modelling of migrating cells and angiogenesis”

11.40 - 12.00 María José Oliveira, University of Porto, Portugal
“Decellularized human colorectal cancer matrices as a tumor microenvironment biomimetic model”

12.00 – 14.00 Lunch

Justiça e Paz, Couraça de Lisboa, 30

Keynote Speaker

14.00 - 14.40 Lino Ferreira, University of Coimbra, Portugal
“Mechanical forces in vascular cell maturation and disease”
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.40</td>
<td>Mechanotransduction and Cytoskeleton</td>
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<td>14.40</td>
<td><strong>Mirjana Liovic</strong>, Medical Center for Molecular Biology, Institute for Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia</td>
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<td>14.40</td>
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<td>“Mechanomodulation of human stem cells: a crosstalk between cytoskeleton and nucleus”</td>
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<td><strong>Andreja Ambriović-Ristov</strong>, Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia</td>
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<td>“The αv-integrin adhesome affects melanoma cell migration and sensitivity to microtubule poisons”</td>
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<td>15.40</td>
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<td>15.40</td>
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<td>16.00</td>
<td>COFFEE BREAK</td>
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<tr>
<td>16.00</td>
<td>New and State-of-the-Art Technological Approaches – Part I</td>
</tr>
<tr>
<td>16.45</td>
<td><strong>Špela Zemljič Jokhadar</strong>, University of Ljubljana, Slovenia</td>
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<tr>
<td>16.45</td>
<td>“Manipulation and analysis of biological samples with optical tweezers”</td>
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<td>17.00</td>
<td><strong>Pedro Melo</strong>, University of Porto, Portugal</td>
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<td>17.00</td>
<td>“Live cell imaging sheds light on the mechanical principles of microglia activation”</td>
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<td>17.15</td>
<td><strong>Irina Hein</strong>, IBIDI GmbH, Martinsried, Germany</td>
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<td>17.15</td>
<td>“An advanced tool for a direct End-Point chemotaxis assay”</td>
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<td>17.30</td>
<td>Concluding Remarks and Discussion</td>
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<td>17.30</td>
<td>Time for interaction and networking</td>
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<tr>
<td>17.30</td>
<td>Networking Dinner</td>
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<td>19.30</td>
<td><strong>Caves do Conde, Rua Adelino de Veiga, 39</strong></td>
</tr>
</tbody>
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Tuesday, 2\textsuperscript{nd} April

Keynote Speaker

10.00 – 10.40 **Maddy Parsons**, Randall Centre for Cell and Molecular Biophysics, King’s College London, UK

“Molecular mechanisms controlling cytoskeletal dynamics”

Redox Regulation/Modulation and Cell Migration  
Session Chair: Rui Travasso

10.40 – 11.00 **Nuno Saraiva**, CBIOS, Universidade Lusófona Research Center for Biosciences & Health Technologies, Lisbon, Portugal

“The role of a new family of Golgi ion channels on cell migration”

11.00 - 11.20 **Ana S. Fernandes**, CBIOS, Universidade Lusófona Research Center for Biosciences & Health Technologies, Lisbon, Portugal

“Modulation of cancer cell migration by redox-active compounds”

11.20 - 11.40 **Armindo Salvador**, University of Coimbra, Portugal

“Hydrogen peroxide signaling in the cytoplasm of eukaryotic cells: what mechanisms are viable?”

12.00 – 14.00 Lunch

*Justiça e Paz, Couraça de Lisboa, 30*

Keynote Speaker

14.00 - 14.40 **Inês Mendes Pinto**, International Iberian Nanotechnology Laboratory, Portugal

“Dimensionality in cell dynamics”

Mechanobiology and Aging  
Session Chair: Juan Carlos Rodríguez-Manzaneque

14.40 - 15.00 **Cláudia Cavadas**, University of Coimbra, Portugal

“Autophagy and Aging”

15.00 - 15.20 **Yannis Missirlis**, University of Patras, Patras, Greece

“Vascular Tissue Engineering: basic principles for proper cell’s function”
15.20 - 15.40 Špela Zemljič Jokhadar, University of Ljubljana, Slovenia
"Mechanical properties of MDA-MB-231 cells treated with metformin and 2-deoxy glucose"

15.40 - 16.15 COFFEE BREAK

New and State-of-the-Art Technological Approaches – Part II

16.15 – 16.35 Ramunas Valiokas, Department of Nanoengineering, Center for Physical Sciences and Technology, Vilnius, Lithuania
"Cellular networks and cell co-culture systems on chemically cross-linked biomimetic hydrogels"

16.35 – 16.50 Heloísa Gerardo, Center for Neuroscience and Cell Biology, University of Coimbra, Cantanhede, Portugal
"Fabrication and functionalization of cell culture substrates for mechanobiology studies"

16.50 – 17.05 Cristina Barrias, University of Porto, Portugal
"Molecularly-designed hydrogels as cell-instructive 3D microenvironments for tissue engineering and cancer research"

17.05 – 17.20 Mirjana Liovic, Medical Center for Molecular Biology, Institute for Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
"Induced pluripotent stem cell (iPSC) line from an epidermolysis bullosa simplex patient heterozygous for keratin 5 E475G mutation and with the Dowling Meara phenotype"

17.20 - 17.35 Célia Aveleira, Center for Neuroscience and Cell Biology, University of Coimbra, Portugal
"How to monitor autophagy in biological systems?"

Wrap Up Session

17.35 Time for discussion and approach conclusions of the meeting and future actions

19.30 Networking Dinner
ABSTRACTS
Epithelial plasticity in health and disease (the Ins and Outs of the EMT)

M. Ángela Nieto
Instituto de Neurociencias (CSIC-UMH), Alicante, Spain anieto@umh.es

Epithelial homeostasis is crucial to maintain tissue architecture, and therefore, it needs to be tightly regulated in the adult. By contrast, embryonic cells show a high degree of epithelial plasticity required for proper morphogenesis and, in particular, for the implementation of massive cell movements that occur during gastrulation and neural crest delamination among other processes. We have been interested in the analysis of cell movements, plasticity and epithelial to mesenchymal transitions (EMT) for many years, and found that the reactivation of developmental EMT-like programs in adult cells leads to several pathologies including tumor progression and organ degeneration. While the epithelial and mesenchymal cells are usually considered as extreme phenotypes, intermediate EMT states also exist. Under those circumstances cells depict a hybrid phenotype expressing both epithelial and mesenchymal markers and from which they can reverse to the original state or move towards a more mesenchymal phenotype. Hybrid transitory states can favor coordinated cell migration or wound healing but they can also enable the formation of clusters of migratory cancer cells with increased metastatic potential. However, in contrast to the situation in cancer, the intermediate phenotype holds promise for new antifibrotic therapeutic approaches, as inhibiting EMT can attenuate established fibrosis. I will discuss different scenarios in which this intermediate phenotype is observed both in development and in disease, frame it in the context of debates in the field and refer to a new developmental EMT that we have found to be crucial for heart laterality and morphogenesis in vertebrates.

References:
Ocaña et al., Cancer Cell (2012)
Nieto et al, Cell (2016)
Brabletz et al., Nat Rev Cancer (2018)
In blood vessels, biomechanical strains induce biological changes in vascular smooth muscles (SMCs) and endothelial cells (ECs) via mechanotransduction. Whereas ECs are primarily exposed to fluid shear stress, SMCs are mainly exposed to cyclic biomechanical stretch, which plays a key role in controlling the tone of the vessel and concomitant blood pressure. During my presentation, I will give some examples about the importance of flow shear stress as well as biomechanical stretch to mature vascular cells and I will demonstrate the importance of mechanical forces in the phenotype of a specific vascular ageing disease called Hutchinson-Gilford Progeria Syndrome (HGPS). HGPS is a premature aging disease in children that leads to early death. Smooth muscle cells (SMCs) are the most affected cells in HGPS patients, although the reason for such vulnerability remains poorly understood. During my presentation, I will show some of our experimental results regarding the effect of mechanical forces in smooth muscle cells.
KEYNOTE SPEAKER

_Molecular mechanisms controlling cytoskeletal dynamics_

Karin Pfisterer, James Levitt, Richard Marsh, Susan Cox, Simon Ameer-Beg and Maddy Parsons

Randall Centre for Cell and Molecular Biophysics, King’s College London, Guys Campus, London, SE1 1UL, UK

Cells have to dynamically adapt their cell shape to respond to changes in the extracellular environment. This is a key feature of cells in many homeostatic and pathological situations, ranging from embryonic development and differentiation to wound healing and metastatic cancer cell migration. Changes in cell shape require dynamic reorganisation of the F-actin cytoskeleton. Fascin is a highly conserved F-actin bundling protein that plays a key role in controlling filopodia stability and adhesion dynamics in migrating cells. Fascin is also very highly upregulated in human cancers, correlates with poor clinical prognosis and metastasis, and is required for efficient cancer cell invasion. However, the mechanisms governing spatial, temporal and functional regulation of fascin in living cells remains poorly understood. Our combined FRAP, high-speed time domain FLIM and 2-color single molecule imaging of fascin dynamics and interactions in live human cancer cells reveals highly localised rapid molecular shuttling of fascin within filopodia correlating with fascin-actin interaction kinetics. We further show that fascin kinetics depend on substrate stiffness in both 2D and 3D matrices. Our studies shed light on the molecular control of fascin activity within tumour cells and may provide new therapeutic targets to halt cancer progression.
Recent studies have highlighted the distinctive morphology and dynamics of cells cultured in two-dimensional (2D) and three-dimensional (3D) microenvironments. However, the mechanical principles underlying cell shape and movement in different dimensions remains largely unknown. Using a combination of computational modelling and quantitative imaging, Inês Mendes Pinto seminar will focus on the interplay between contractile and adhesive forces in determining cell behaviour in 2D and 3D microenvironments.
**ECM AND CELL MIGRATION**

*Computational modelling and experimental approaches identify a role of ECM stiffening in SRC-induced EMT*

Florence Janody
Instituto de Investigação e Inovação em Saúde (I3S), University of Porto, Portugal

Epithelial to Mesenchymal Transition (EMT) has been associated with the acquisition of migrating and metastasis abilities as well as of cancer stem-like features in a wide variety of human carcinoma. Research has merely highlighted how complex this phenomenon is in cancer, leaving many exciting open questions on how this process is coordinated by many signalling pathways and by cues from the microenvironment.

To understand the series of events leading to this phenotypic transition, we defined a logical model of the EMT cellular network controlled by signals coming from the microenvironment. As read out of the epithelial and mesenchymal states, the network displays diverse degrees of cell adhesion properties by adherent junctions (AJs) and focal adhesions (FAs). Model attractors predict that in addition to purely epithelial or purely mesenchymal states, 6 partial EMT stable states can be recovered, including a hybrid state, which maintains AJs, while acquiring dynamic FAs turnover. The model indicates that EMT can be achieved through various trajectories involving sequential molecular changes, which depend on distinct inputs from the microenvironment and on-going through intermediate EMT states. Microenvironment. Of particular interest, the model predicts that 1) ECM stiffening is an obligate requirement for cells overactivating FAK-SRC to acquire a full mesenchymal phenotype and that 2) the inhibition of the type IIb family of Receptor Protein Tyrosine Phosphates (RPTP) by FAK-SRC prevents the acquisition of a hybrid phenotype, while endorsing a full mesenchymal phenotype. Experimental validation using the human mammary epithelial cell line MCF10A with conditional SRC activation indicates that on a stiff substrate, SRC downregulates PTPRK, the gene that encodes for RPTP-k, prior to acquiring mesenchymal morphologies. Moreover, preventing the downregulation of RPTP-k by SRC restores cell-cell adhesions.

Alltogether, our combined computational and experimental approaches permitted to identify critical microenvironmental inputs involved in EMT and suggests that EMT in the context of cancer might involve multiple molecular programs rather than a unique one.
The contribution of extracellular proteases for cell invasion and migration was already introduced more than a decade ago, mainly for MMPs (matrix metalloproteinases). The later discovery of a large number of proteases of the closely related families ADAMs (a disintegrin and metalloprotease) and ADAMTSs (a disintegrin and metalloprotease with thrombospondin motifs) remarked the necessity to approach deeper investigations. Recent studies on tumor heterogeneity are including the importance of proteolytic remodeling for the dynamism of extracellular components, regulating angiogenesis, metastasis, invasive phenotypes, and also the infiltration of inflammatory cells. Our studies with the protease ADAMTS1 disclosed its anti-angiogenic activities but also its tumorigenic and metastatic properties, highlighting its microenvironment-dependent actions. For example, the identification of various substrates of ADAMTS1 has been directly associated with invasive and migratory phenotypes. More recently, our work with syngeneic tumorigenic models, using B16F1 and LLC murine cancer cells in wild type and Adamts1 knockout mice, exposed the alteration of relevant vascular parameters in both models although with distinct consequences for tumor progression. Indeed, our findings supported a pro-tumorigenic contribution of stroma ADAMTS1 in B16F1 melanomas. A deeper characterization of these tumors showed a distinctive infiltration of macrophage and immune-related cells depending on the presence or absence of the protease. Moreover, the implication of the substrate Nidogen-1 has also been observed, primarily by an altered deposition on the vasculature, so our research is now focused to unveil related players in the extracellular microenvironment with impact to restrain final tumor growth.
Biochemical processes regulate extracellular matrix (ecm) remodelling, actin polymerisation, membrane deformation and cell-ecm adhesion. This permits for cells to exert forces in the ecm, and to migrate and/or to rearrange themselves into complex structures. For example, blood vessel formation and blood vessel remodelling are processes where biochemical mechanisms are intertwined with vessel mechanics, tissue mechanics, growth factor diffusion, and matrix mechanical properties and degradation. In this talk I will show how to construct mathematical models of cell migration and vessel formation by taking into account this mechanical interplay between the cells and their microenvironment. I will focus on the ability for these models to suggest testable hypothesis and to provide new insights into the mechanisms that drive the dynamics in these biological systems.
ECM AND CELL MIGRATION

*Decellularized human colorectal cancer matrices as a tumor microenvironment biomimetic model*


Instituto de Investigação e Inovação em Saúde (I3S), University of Porto, Portugal

In the present work, we investigated the impact of human colorectal tumor matrices on macrophage polarization and macrophage-mediated cancer cell invasion. Accordingly, we developed an innovative 3D-organotypic model, based on the decellularization of normal and tumor tissues derived from colorectal cancer patients' surgical resections. Extensive characterization of these scaffolds revealed that DNA and other cells constituents were efficiently removed, while native tissue characteristics, namely major ECM components, architecture and mechanical properties, were preserved.

Notably, normal and tumor matrices distinctly promoted macrophage polarization, with macrophages in tumor matrices differentiating towards an anti-inflammatory M2-like phenotype (higher IL-10, TGF-β, and CCL18 and lower CCR7 and TNF expression). Matrigel invasion assays revealed that tumor ECM-educated macrophages efficiently stimulated cancer cell invasion through a mechanism involving CCL18. Notably, the high expression of this immunosuppressive chemokine at the invasive front of human colorectal tumors associated with advanced tumor staging. Notably, this work demonstrated that decellularized matrices constitute excellent scaffolds when trying to recreate complex microenvironment to understand basic mechanisms of disease or therapeutic resistance.
MECHANOTRANSDUCTION AND CYTOSKELETON

Cytoskeletal mutations and keratinocyte dynamics and elasticity

Jure Dergane1, Marcos Gouveia2, Biljana Stojković1, Marko Vidak3, Špela Zemljič-Jokhadar1, Rui D.M. Travasso2, Mirjana Liovic3

1 Institute for Biophysics, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia; 2 Centro de Física da Universidade de Coimbra (CFisUC), Department of Physics, University of Coimbra, R. Larga, 3004-516 Coimbra, Portugal; 3 Medical Center for Molecular Biology, Institute for Biochemistry, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia.

Keratins are intermediate filament proteins (IF) expressed in all epithelial cells. Keratin gene mutations cause a number of severe hereditary skin fragility disorders. Amongst these is the simplex subtype of epidermolysis bullosa (EBS), linked to mutations in keratins 5 and 14. Mutant keratins disrupt the intermediate filament cytoskeleton and place EBS keratinocytes in a state of stress. This causes a continuous activation of MAP kinase signaling pathways and alters gene expression of hundreds of other proteins, many of which have a function in cell–cell and cell-surface adhesion. In this talk I will present some of the findings of past and ongoing work related to the dynamic keratin particles that are often observed at the periphery of keratin 5 and 14 mutant keratinocytes. I will also present the new mathematical model of keratin turnover in wild type and mutant cells, where we assumed that keratin mutations cause a slowdown in the assembly of an intermediate (keratin) phase into filaments. Experiments with optical tweezers showed that the altered keratin distribution affects the stiffness of the cell cortex, and found that mutant cells exhibited a higher cortical stiffness than wild type cells. This correlates with the prediction of our mathematical model, where an increased concentration of non-soluble keratin forms at the cell cortex.
MECHANOTRANSDUCTION AND CYTOSKELETON

Mechanomodulation of human stem cells: a crosstalk between cytoskeleton and nucleus.

Heloísa Gerardo¹, Catarina Domingues¹, Ana Lima¹², Margarida Geraldo¹, João Carvalho², Sandra I. Anjo¹, João R. D. Ramos³, Sofia Couceiro³, Rui D. M. Travasso³, Bruno Manadas¹, Ricardo Pires das Neves¹⁶,∗ & Mário Grãos¹⁶⁷,∗

¹ CNC — Center for Neuroscience and Cell Biology, University of Coimbra, UC-Biotech Building, Biocant Park, Cantanhede, Portugal; ² Faculty of Science and Technology, University Nova of Lisbon (MIT-Portugal PhD Program), Caparica, Portugal; ³ Centro de Fisica da Universidade de Coimbra (CFisUC), Department of Physics, University of Coimbra, Coimbra, Portugal; ⁴ Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany; ⁵ Stemlab S.A. (Crioestaminal), Biocant Park, Cantanhede, Portugal; ⁶ Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal; ⁷ Biocant, Technology Transfer Association, Cantanhede, Portugal; ∗ R.P.N. and M.G. are co-senior authors in this study.

Increasing evidence has shown that the biophysical properties of the extracellular environment influence, to a large extent, gene expression and consequently cell-fate decisions. It is becoming apparent that such mechanical stimuli can be transduced from the extracellular matrix (ECM) to the nucleus in a direct physical manner (through the continuous multi-protein network present between the ECM and the nucleus), as well as by mechanosensitive biochemical signal transduction cascades that ultimately regulate molecular transcriptional modulators, in both cases resulting in regulation of gene expression. Here, we present two distinct cellular systems in which the aforementioned mechanisms seem to play a significant role, highlighting the importance of intracellular contractility and the actin cytoskeleton for the regulation of transcription and cell fate decisions.

Cellular stemness is intimately related with the mechanical status of the cell, such as intracellular contractility and cellular stiffness, which in turn are influenced by the microenvironment. Pluripotent stem cells are typically soft, displaying low intracellular contractility and highly deformable nuclei. Because cellular stiffness, actomyosin contractility and nuclear pre-stress scale directly with substrate/matrix rigidity, we postulate that soft cell culture substrates can lead to an overall intracellular relaxed state, hence contributing to increased reprogramming efficiency of human umbilical cord mesenchymal stem/stromal cells (hUC-MSCs) into induced pluripotent stem cells (iPSCs). We demonstrate that soft substrates (1.5-15kPa) can modulate several cellular features of MSCs into a phenotype closer to pluripotent stem cells (PSCs). MSCs cultured on soft substrates present more relaxed nuclei, lower maturation of focal adhesions and F-actin assembling, more euchromatic and less heterochromatic nuclear DNA regions, and increased expression of pluripotency-related genes than those on stiff (GPa) substrates. These changes correlate with the reprogramming of MSCs, with a positive impact on the kinetics, robustness of colony formation and reprogramming efficiency, which is supported by a mathematical model predicting that low traction force favors full reprogramming to pluripotency. Additionally, substrate stiffness influences several phenotypic features of iPSC cells and colonies.

On a distinct dataset, which includes an unbiased quantitative proteomics approach (SWATH-MS), we identified a novel mechanotransduction player that scales inversely with substrate rigidity or actomyosin tension. We also demonstrated that this protein shows a preferred nuclear or cytosolic localization when naïve hUC-MSCs are cultured on soft (~3kPa) versus stiff (GPa range) substrates, and similar results were obtained during pharmacological induction of low or high actomyosin-mediated tension (respectively), regardless of substrate stiffness. This indicates that actomyosin contractility plays a major role in regulating both the abundance of the protein and its intracellular localization. Finally, by using both pharmacological and molecular approaches (e.g. siRNA), we demonstrate that the nuclear localization of the protein, achieved by controlling substrate stiffness or intracellular tension, correlates with an apparently generalized increase in nuclear gene transcription.
MECHANOTRANSDUCTION AND CYTOSKELETON

The αv-integrin adhesome affects melanoma cell migration and sensitivity to microtubule poisons

M. Paradžik¹, J.D. Humphries², A. Dekanić³, D. Nestić³, D. Majhen¹, N. Stojanović¹, D. Sedda¹, I. Samaržija¹, I. Weber³, M.J. Humphries², Andreja Ambriović-Ristov¹

¹Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruder Bošković Institute, Zagreb, Croatia; ²Wellcome Trust Centre for Cell-Matrix Research, Faculty of Biology, Medicine & Health, University of Manchester, Manchester, United Kingdom; ³Laboratory for Cell Biophysics, Division of Molecular Biology, Ruder Bošković Institute, Zagreb, Croatia

Integrins are heterodimeric glycoproteins that bind cells to extracellular matrix proteins. Upon integrin clustering, multimolecular integrin adhesion complexes (IACs) are formed, facilitating the linkage between integrins and the actin cytoskeleton and permitting bidirectional signalling. To better understand the previously observed change in cell migration and sensitivity to microtubule poisons upon transient transfection with integrin αV-specific siRNA, the aim of this work was to assess αV-dependent changes in IAC composition in two melanoma cell lines MDA-MB-435S and RPMI-7951. Two MDA-MB-435S-derived integrin αV-specific shRNA expressing cell clones, with decreased expression of integrin αV, expressing 15% (2αV) or 5% (3αV) of the control cells showed decreased migration and increased sensitivity to paclitaxel and vincristine. These results are consistent with previous results obtained following transient transfection with integrin αV-specific siRNA. Similarly, knockdown of integrin αV in the melanoma cell line RPMI-7951 using integrin αV-specific siRNA (RPMI-7951/si(αV)) decreased migration and increased sensitivity to paclitaxel and vincristine compared to cells transfected with the control siRNA (RPMI-7951/si(αV)). In both cell models the decreased expression of integrin αV influenced cell morphology, i.e. the cells were smaller than the control cells and had a lower number of focal adhesions as observed by interference reflection microscopy and immunofluorescence detection of phosphopaxillin. The molecular composition of isolated IACs from MDA-MB-435S cells and cell clones 2αV and 3αV, as well as from RPMI-7951/si(αV) and RPMI-7951/si(αV) cells, was analysed using mass spectrometry (MS)–based proteomics. MS analysis from MDA-MB-435S and RPMI-7951/si(αV) cell lines showed that these cells preferentially use integrin αVβ5 for the formation of IAC. As expected, in clones 2αV and 3αV or RPMI-7951/si(αV), integrins αV and β5 were detected at much lower levels compared to control cells. When clones 2αV and 3αV were compared to MDA-MB-435S cells or RPMI-7951/si(αV) compared to RPMI-7951/si(αV), lower levels of talin-2, alpha-actinin-1 and -4, filamin-A and -B, plectin and vinculin were detected. These data will enable follow-up analyses of signalling mediated by integrin αVβ5 and therefore represent a valuable resource to improve our understanding of the mechanisms involved adhesion control of melanoma cell sensitivity to microtubule poisons and cell migration.
MECHANOTRANSDUCTION AND CYTOSKELETON

Mechanotransduction regulates endothelial cell behavior in an in vitro blood brain barrier model

Ece Bayir, M. Mert Celtikoglu, Aylin Sendemir
Ege University, Izmir, Turkey

There are several blood-brain barrier (BBB) models frequently used in drug development, screening, and targeting studies, but none of those are able to properly replicate the physiological permeabilities of this special barrier.

The aim of this study is the construction of a reliable in vitro BBB model mimicking physiological BBB characteristics, in terms of cellular organization and mechanobiology.

Bacterial cellulose (BC) is chosen as a basement membrane. Because of BC’s nano-porous structure, the nutrient transfer is enabled, while cell migration is restricted. BC was produced both in sheet and vessel forms, for static and dynamic cultivation, respectively.

For the dynamic model, BBB bioreactor system was designed and constructed in order to simulate the physiological blood flow and shear stress in brain capillaries.

Human brain microvascular endothelial cells (HBMECs) were seeded in luminal section and astrocytes and human brain microvascular pericytes (HBMPCs) were seeded in the abluminal section, and were cultivated together without changing compartments.

Cell viability was observed by Live&Dead staining. Immunofluorescence (IF) staining was performed in order to observe specific markers of the cells, and scanning electron microscopy (SEM) was performed in order to observe morphology of the cells.

Caffeine and sucrose permeabilities of the models were determined by HPLC analysis, and trans-endothelial electrical resistance (TEER) values were measured by epithelial volt/ohm meter. The expression levels of tight and adherence junction proteins of HBMECs were analyzed by quantitative polymerase chain reaction (qPCR).

This work has been supported by the Scientific and Technological Research Council of Turkey (TUBITAK) through Project no: 216M542.
NEW AND STATE-OF-THE-ART TECHNOLOGICAL APPROACHES - PART I

Manipulation and analysis of biological samples with optical tweezers

Špela Zemljič Jokhadar, Jure Derganc
University of Ljubljana, Slovenia

Optical tweezers (OT) are formed by a highly focused laser beam and are used for manipulation of nano- and micro-sized objects, like microspheres, vesicles or whole cells. The focused laser beam attracts objects, traps them and holds them by a pN force. The technique can be applied on living cells, since it is non-invasive and it operates in a non-contact mode. In cell biology it can be applied for measuring different mechanical features of the cells, for example cell stiffness, the force needed for the separation of the plasma membrane and the underlying cytoskeleton, the availability of the plasma membrane reservoir, the adhesiveness of the plasma membrane,… OT proved to be a useful method for mechanical analysis of single cells and is complementary to other methods of this kind as atomic force microscopy for example.
Live cell imaging sheds light on the mechanical principles of microglia activation

Melo, Pedro$^{1,2}$, Mendes-Pinto, Inês$^2$, Relvas, João$^1$

$^1$ Glial Cell Biology Group, Instituto de Investigação e Inovação em Saúde (I3S), Porto, Portugal; $^2$ Cell Mechanics Laboratory, International Iberian Nanotechnology Institute (INL), Braga, Portugal

Microglia, the main central nervous system innate immune cells, undergo shape and motility changes necessary for their functions, such as acquisition of directed motility, increase in phagocytic activity and secretion of inflammatory mediators. Upon activation, they typically lose their elongated morphology in favour of an amoeboid shape. This makes them particularly interesting for studying how cytoskeleton reorganization can contribute to the specific functions of a cell. Live cell microscopy and the application of techniques like fluorescent speckle microscopy and FRET are uniquely suited to the study of cytoskeleton dynamics and intracellular signalling pathways, revealing detailed information about the kinetics and spatiotemporal distribution of events inside a cell. We have employed such techniques to study the behaviour of a human microglia cell line, and to quantitatively determine the distribution of actin and myosin motors in response to inflammatory stimulation. Our results revealed a transition in the signal topography of actomyosin from a symmetric, non-polarized distribution under steady state conditions, to an asymmetric, polarized distribution upon activation. We propose that microglial inflammatory activation causes myosin II motors to cluster to a region of the cortex, pulling the actin network towards it and allowing acquisition of the classical microglia amoeboid shape.
An Advanced Tool for a Direct End-Point Chemotaxis Assay

This work will be presented by Irina Hein\textsuperscript{1} based on the scientific work of Lea Tomasova\textsuperscript{1*}, Zeno Guttenberg\textsuperscript{1}, Bernd Hoffmann\textsuperscript{2} and Rudolf Merkel\textsuperscript{2}

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Chemotaxis of slow migrating cells plays a crucial role in numerous physiological and pathophysiological processes such as embryonic development, wound healing and tumor metastasis. The state-of-the-art chemotaxis assays are in general designed either with respect to the migration characteristics of fast-moving cells, or focus on an in-depth investigation of chemotactic behaviour, requiring a time-demanding and labor-intensive analysis of individual cell trajectories. This is rather inefficient for applications requiring higher experimental throughput, e.g. clinical examinations or chemoattractant screenings. Here we present an advanced migration assay for accelerated and facilitated evaluation of the chemotactic response of slow-moving cells. The revised chemotaxis chamber contains a microstructure-based migration arena, designed to enable fast and effortless analysis of chemotaxis in respect to the end-point experiment. The assay in form of a microscopy slide enables direct visualization of the cells in either 2D or 3D environment, and provides a stable and linear gradient of chemoattractant. Taken together, this advanced tool substantially facilitates the analysis, providing for an increased experimental throughput.
How to monitor autophagy in biological systems?

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Autophagy, a highly regulated intracellular process involved in the turnover of most cellular constituents and in the maintenance of cellular homeostasis. Autophagy has been implicated in many physiological and pathological processes, namely development, aging, immunity, cancer, metabolic and neurodegenerative diseases. It has also been shown to play a role in cell differentiation, migration, survival and death.

Autophagy is a process in which proteins, protein oligomers, other macromolecules, and even organelles, are first engulfed by specialized double membrane vesicles, termed autophagosomes. These autophagosomes are then transported along microtubules towards the microtubule organizing center of cells, where the lysosomes are clustered. After fusion and content exchange with lysosomes, the autophagosome cargo is degraded by lysosomal hydrolases. The constant flow of autophagosomes to lysosomes is tightly regulated by several signaling pathways.

Given its dynamic and complex nature, there is a need to accurately identify, quantify and manipulate the autophagic process.

In our laboratory, we provide state-of-the-art methodology to monitor autophagy and modulate autophagic activity in several biological systems. We perform specialized techniques for the analysis of autophagic pathways, including immunoblotting, light microscopy, live-imaging assays, and electron microscopy. We also provide advice on experimental design, techniques, and data interpretation. Autophagy assays should generally not be performed alone, but should be accompanied by complementary assays to enable robust interpretations.

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REDOX REGULATION/MODULATION AND CELL MIGRATION

The role of a new family of Golgi ion channels on cell invasion

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The mechanisms underlying the Golgi apparatus impact on cell invasion are still poorly understood. The human Golgi anti-apoptotic protein (hGAAP) is a novel, highly conserved Golgi cation channel that modulates intracellular Ca²⁺ fluxes. hGAAP is expressed in all human tissues, is essential for cell viability and provides resistance against a range of apoptotic stresses. Furthermore, hGAAP enhances cell adhesion and 2-dimensional random cell migration by increasing the turnover of focal adhesions due to activation of store-operated Ca²⁺ entry. Bioinformatics analyses suggest a link between dysregulation of hGAAP expression and several human cancers.

We describe a novel Golgi apparatus-derived mechanism that controls cell invasion. The overexpression of hGAAP strongly stimulates in vitro and in vivo 3D proteolytic cell invasion by a mechanism that is dependent on the accumulation of intracellular H₂O₂ likely produced by the stimulation of mitochondrial respiration. A deeper understanding of the impact of hGAAP on cell invasion and cell metabolism will provide new insights into the complex mechanisms related to Ca²⁺ and ROS signaling in the context of cell invasion and will contribute to elucidate the role of the Golgi apparatus in these events.
REDOX REGULATION/MODULATION AND CELL MIGRATION

Modulation of cancer cell migration by redox-active compounds

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The role of reactive species (RS) as signaling players during cancer cell migration and, consequently, in metastases is becoming increasingly apparent. Both O2− and H2O2 levels seem to be implicated in the regulation of cancer cell migration. However, the underlying regulation mechanisms are only beginning to be elucidated and contradictory results about the effect of RS and antioxidants can be found in the literature. In this work, we evaluated the effects of redox-active compounds, either of synthetic or natural origin, in the motility of breast and renal cancer cells. Manganese(III) porphyrins (MnP) are synthetic antioxidants that mimic superoxide dismutase, scavenge different RS and modulate redox signaling. MnPs are currently in clinical trials in patients submitted to chemo- or radiotherapy, due to their ability to boost anticancer treatments while protecting off-target tissues. Although RS are implicated in the metastatic process, only scarce studies have addressed the impact of MnPs in metastases. Herein we characterized the impact of non-cytotoxic levels of an MnP (MnTnHex-2-PyP⁵⁺) in metastases-related processes. This MnP was studied in MCF7 and MDA-MB-231 breast cancer cells alone and in combination with doxorubicin (dox). The co-treatment decreased the collective motility of MCF7, the chemotactic migration of both cell lines, and the proteolytic invasion of MDA-MB-231 cells. MnP also counteracted the increase in random MDA-MB-231 cell migration induced by dox. To explore the underlying mechanisms, the effects in cell spread/area, focal adhesions, intracellular RS levels, and NFκB activity were studied. In renal cancer cells 786-O, this MnP significantly decreased chemotaxis. In addition, we have studied two structurally distinct redox-active dietary compounds using the same cell model: thymoquinone and erucin. Previous data suggest that these bioactive compounds might have anticancer properties, but only scarce studies addressed their impact on renal cancer cells motility. Overall, our results suggest that redox-active compounds may have a beneficial impact in reducing cancer cells migration and warrant further studies regarding novel anticancer therapeutic approaches.

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Hydrogen peroxide signalling in the cytoplasm of eukaryotic cells: what mechanisms are viable?

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Hydrogen peroxide (H2O2) is a well-established signaling agent in cell migration, proliferation, and apoptosis, but its signaling mechanisms remain poorly understood. Its best characterized signaling actions involve the oxidation of thiols in cytoplasmic phosphatases, kinases and transcription factors within minutes of cells' stimulation. How such a fast oxidation can occur in presence of extremely abundant and H2O2-reactive cytoplasmic peroxiredoxins is a key open question. We have quantitatively assessed the plausibility of a series of alternative hypotheses to explain this phenomenon using mathematical modeling of the H2O2/peroxiredoxin/thioredoxin system. The results suggest that H2O2 signaling under resting (i.e. non-oxidative stress) is mediated by localized redox relays whereby peroxiredoxins are oxidized to sulfenate and disulfide forms at H2O2 supply sites and these forms in turn oxidize the redox targets near these sites.
Autophagy is a highly conserved cellular process that autophagy is a constitutive lysosomal catabolic pathway that degrades damaged organelles and protein aggregates. As a main intracellular degradation and recycling pathway, autophagy is critical for maintaining cellular homeostasis, as well as for remodeling during normal development. Impairment of Autophagy has been implicated in various diseases and also in aging. Autophagy impairment and also stem cells exhaustion are two of the nine hallmarks of aging, contributing to the aging phenotype and to the aggravation of age-related diseases. Strategies that promote autophagy and delay stem cells exhaustion are relevant to control the aging process. NPY (neuropeptide Y) is an endogenous neuropeptide that stimulates stem cells proliferation and autophagy in different brain areas. Moreover, NPY mediates caloric restriction-induced autophagy in hypothalamic neurons. Since both stem cells and autophagy decrease with age, modulation of NPY levels could provide new putative therapeutic tools to ameliorate age-related deteriorations and extend longevity.

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MECHANOBIOLOGY AND AGING

Vascular tissue engineering: basic principles for proper cell’s functions

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There have been many important advances in the design and production of small caliber vascular grafts in recent years. The main approaches are either scaffold-based or self-assembly involving bioprinting. An innovation process has been the so-called dynamic maturation of the graft before implantation. However latency is still missing.

Here some information is presented on the response of endothelial cells cultured on cylindrical small diameter tubes under a combination of mechanical cues. Morphological and gene responses are presented. The approach is amenable to a more sophisticated setup where also fibroblasts and vascular smooth muscle cells will be incorporated.
Mechanical properties of MDA-MB-231 cells treated with metformin and 2-deoxy glucose

Maruša Bizjak, Anja Kraševček, Špela Zemljič Jokhadar, Jure Derganc, Mojca Pavlin
University of Ljubljana, Slovenia

In the process of metastasization circulating cancer cells migrate through the capillary wall to new sites in the body, re-attach and proliferate. To do so they must resist anoikis, which is a programmed cell death induced by detachment from the extracellular matrix. The triple negative breast cancer is known for extensive metastasization. The most commonly used cell line model for this kind of breast cancer is MDA-MB-231. In previous studies we assessed the anti-proliferative effects of metformin and 2-deoxy glucose (2-DG) two known anti-cancer agents on MDA-MB-231 cells. The results from these experiments showed that a combined treatment with 5mM metformin and 600 μM 2-DG completely blocks proliferation of MDA-MB-231 cells but it also increases the fraction of floating (un-attached) cells from which about 71 % were viable. These finding was surprising and we decide to look at it more carefully as we could not find any reports regarding this phenomena. Among others we will look at the mechanical aspect, so we will measure the cell stiffness of control cells and cells treated for 24 and 48 hours with optical tweezers. We also looked at the organization and distribution of F-actin, β1 integrin and paxillin as a principal determinant of focal adhesions. The main goal is to determine, if the treated cells will also change mechanically in a way, which would theoretically help them to invade new tissues.
NEW AND STATE-OF-THE-ART TECHNOLOGICAL APPROACHES - PART II

Cellular networks and cell co-culture systems on chemically cross-linked biomimetic hydrogels

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Despite the huge variety of available hydrogel biomaterials, very few of them display the elastomechanical properties required to fabricate self-supporting structures and devices for advanced cell biology and tissue engineering applications. In our work, we have been focusing on the development of chemically crosslinked materials that beside biocompatibility have controlled compositional and structural integrity both in cell culture and in vivo. This presentation will provide a survey of the main application directions of such hydrogel-based systems that we have successfully integrated with functional surface patterning and topographical fabrication. We have been capable to fabricate cell-adhesive features on either bio-inert or tissue-regenerating coatings/material blocks for docking of single-cells, cellular networking and formation of minimalistic tissue models. The feature size and shape can be controlled with nanometer precision for, e.g. definition of the force vectors in individual cells or for programming of unidirectional cell motion. The developed array structures proved very useful in studies of different tissue systems, including cornea, bone, heart and brain by employing both immortalized and primary cells. Also, the surface topography control provides powerful means to engineer systems for 2,5 and 3D cell culture and, most importantly, for co-culturing of different types of cells. To illustrate the advantages of this approach, the presentation will cover cultivation of primary stem cells as well as assembly of tissue-like constructs consisting of cerebellar cells, skin keratinocytes and fibroblasts.
NEW AND STATE-OF-THE-ART TECHNOLOGICAL APPROACHES - PART II

Fabrication and functionalization of cell culture substrates for mechanobiology studies

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We have established simple and effective methods to produce cell culture substrates for mechanobiology studies. By varying the concentration of polyacrylamide (PAA) and bis-acrylamide, we are able to produce hydrogels with Young’s moduli ranging between ~0.3 and ~10 kPa. Using polydimethylsiloxane (PDMS), we have produced substrates suitable for cell culture in the 1kPa to 20 kPa range ($E'$). Importantly we devised methods to functionalize PAA and PDMS hydrogels, as well as glass coverslips (typically used as a stiff substrate, within the GPa range), which rely on covalent binding of extracellular matrix proteins (or peptides) to allow for efficient and durable cell adhesion. Our protocol also shows improved cell adhesion when functionalizing commercially available PDMS substrates in comparison with the protocol recommended by the manufacturers. So far, we have successfully tested MSCs, iPSCs, oligodendrocytes (both cell lines and primary cells) and neuronal cell lines. In summary, both polyacrylamide and PDMS substrates with defined stiffness (as well as glass coverslips) may be used to covalently bind extracellular matrix proteins (or peptides) that promote efficient cell adhesion and can be used as simple and effective platforms for in vitro mechanobiology studies using distinct cell types.
NEW AND STATE-OF-THE-ART TECHNOLOGICAL APPROACHES - PART II

Molecularly-designed hydrogels as cell-instructive 3D microenvironments for tissue engineering and cancer research

Cristina C. Barrias

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Bioengineered microenvironments can be used to promote 3D cell assembly under controlled conditions. We have established different types of microtissue-systems to recapitulate tissue-specific morphogenesis and differentiation, and to understand the impact of microenvironmental signals in such processes. In this context, we have been developing cell-instructive hydrogels, ranging from complex multifunctional hydrogels, to "minimal matrices" containing only the essential biochemical/biomechanical signals essential for cells to exhibit their unique self-organizing properties. Engineered microtissues provide powerful tools for gaining insight into the mechanisms by which cells perceive their microenvironment to organize into specific structures, and to understand how these processes can be guided by matrix features and/or the presence of other cell types. Ultimately, we aim to translate this knowledge into the design of advanced cell-based regenerative therapies and 3D in vitro models. This presentation will cover some examples of studies we have been conducting using different types of hydrogel-based systems.
We have generated MLi002-A, a new induced pluripotent stem cell (iPSC) line derived from keratinocytes of a skin punch biopsy of a female patient with the severe epidermolysis bullosa simplex Dowling-Meara phenotype and the keratin K5 E475G mutation. Keratinocytes were reprogrammed using non-integrating Sendai virus vectors, and xeno-free culture conditions were used throughout. The characterization of MLi002-A cell line consisted of molecular karyotyping, mutation screening using restriction enzyme digestion and Sanger sequencing, and testing of the pluripotency and differentiation potentials by immunofluorescence of associated markers both in vitro and in vivo. This is the first iPSC model of EB Simplex.
NEW AND STATE-OF-THE-ART TECHNOLOGICAL APPROACHES - PART II

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DETAIL OF COIMBRA’S MAP WITH VENUE AND HOTEL LOCALISATION: