NEW FRONTIERS IN EXTRACELLULAR MATRIX RESEARCH
From regeneration, to immunology, mechanics and soft robotics

ETH Summer School, 9-13 September 2018 | Zurich, Switzerland
Content

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Programme

10-13\textsuperscript{th} September

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<tr>
<td>08:00</td>
<td>Morning coffee</td>
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<tr>
<td>08:30</td>
<td>Opening remarks: The Organizing Committee</td>
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<td>08:45</td>
<td>Keynote Lecture</td>
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<td>Venue: Auditorium Christian Gerber</td>
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<tr>
<td>08:45</td>
<td>“Matrix-Nucleus structural coupling in tissues &amp; mimetics: From the first organ to anti-cancer macrophages”</td>
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<td></td>
<td>Prof. Dennis E. Discher, Ph.D</td>
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<td>Robert D. Bent chaired Professor</td>
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<td>University of Pennsylvania</td>
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<td>USA</td>
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<tr>
<td>10:25</td>
<td>Dr. Eileen Gentleman, Ph.D</td>
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<td></td>
<td>Senior Research Fellow and Principal Investigator</td>
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<td>King’s College London (United Kingdom)</td>
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<td>10:45</td>
<td>Coffee Break</td>
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<tr>
<td>11:25</td>
<td>Prof. Boris Hinz, Ph.D</td>
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<td>Distinguished Professor of Tissue Repair and Regeneration</td>
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<td>University of Toronto (Canada)</td>
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<td>12:00</td>
<td>Dr. Jérome Feige, Ph.D</td>
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<td>Group Head</td>
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<td>Nestlé Institute of Health Sciences (Switzerland)</td>
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<td>12:00</td>
<td>Lunch Break (The Auditorium Foyer)</td>
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<td>14:00</td>
<td>Rapid fire presentations</td>
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<td>15:00</td>
<td>Coffee break and posters sessions</td>
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<tr>
<td>19:00</td>
<td>Dinner</td>
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<td></td>
<td>Restaurant Reithalle (A military horse-riding school turned into modern restaurant. Serves international cuisine). Address: Gessnerallee 8, 8001 Zürich</td>
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### Theme II: Mechano-Matrix
Venue: Auditorium Christian Gerber

Moderated by: Dr. Jens Möller (ETH Zurich)

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<tr>
<td>08:00</td>
<td>Morning coffee</td>
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| 08:30 |                                      09:10 | **Prof. Adam Engler, Ph.D**  
Associate Professor of Bioengineering  
University of California, San Diego (USA) |                                              |
| 09:10 |                                      09:50 | **Prof. Mark Tibbitt, Ph.D**  
Assistant Professor of Macromolecular Engineering  
ETH Zurich (Switzerland) |                                              |
| 09:50 |                                      10:10 | Coffee break and posters session        |                                              |
| 10:10 |                                      10:50 | **Prof. Jess G. Snedeker, Ph.D**  
Associate Professor of Orthopaedic Biomechanics  
University and ETH Zurich (Switzerland) |                                              |
| 10:50 |                                      11:30 | **Prof. Selman Sakar, Ph.D**  
Assistant Professor of Mechanical Engineering  
EPFL (Switzerland) |                                              |
| 11:30 |                                      12:00 | Discussion                           |                                              |
| 12:00 |                                      13:30 | Lunch Break (The Auditorium Foyer)     |                                              |

### Theme III: Immuno-Matrix
Venue: Auditorium Christian Gerber

Moderated by: Dr. Eileen Gentleman (King’s College London)

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| 13:30 |                                      14:10 | **Prof. Kim Midwood, Ph.D**  
Professor of Matrix Biology  
University of Oxford (United Kingdom) |                                              |
| 14:10 |                                      14:50 | **Prof. Andrea Ablasser, MD Ph.D**  
Assistant Professor of Life Sciences  
EPFL (Switzerland) |                                              |
| 14:50 |                                      15:50 | *Group photo #1 >> Coffee break and Posters session* |                                              |
| 15:50 |                                      16:30 | **Prof. Cornelia Halin Winter, Ph.D**  
Associate Professor of Pharmaceutical Immunology  
ETH Zurich (Switzerland) |                                              |
| 16:30 |                                      17:20 | **Prof. Li Tang, Ph.D**  
Assistant Professor of Materials Science  
EPFL (Switzerland) |                                              |
| 19:00 |                                      | Dinner                                      | Griechische Taverne (Family-run restaurant and a piece of Greece in Zurich)  
Address: Seefeldstrasse 167, 8008 Zurich |
### Professional Development Workshops

**Venue:** Balgrist University Hospital
**Rooms 63 and 64**

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<tr>
<td>08:30</td>
<td>Morning coffee</td>
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<tr>
<td>09:00</td>
<td><strong>Scientific Publishing Workshop</strong></td>
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<tr>
<td>09:00</td>
<td><strong>Dr. Kyle Legate</strong></td>
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<tr>
<td>09:00</td>
<td>Senior Editor, <em>Nature Communications</em></td>
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<tr>
<td>10:30</td>
<td>Coffee Break</td>
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<tr>
<td>10:50</td>
<td>Workshop free discussion (Optional: Balgrist</td>
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<td>10:50</td>
<td>Campus tour)</td>
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<tr>
<td>12:00</td>
<td>Lunch Break (The Auditorium Foyer)</td>
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**Theme IV: Responsive-Matrix**

**Venue:** Auditorium Christian Gerber

**Moderated by:** Dr. Xiao-Hua Qin (ETH Zurich)

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<tr>
<td>13:30</td>
<td><strong>Prof. Tal Dvir, Ph.D</strong></td>
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<td>13:30</td>
<td>Associate Professor of Biotechnology, Materials Science &amp;</td>
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<td>13:30</td>
<td>Engineering</td>
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<td>13:30</td>
<td>Tel Aviv University (Israel)</td>
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<td>14:10</td>
<td><strong>Prof. Maartje Bastings, Ph.D</strong></td>
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<td>14:10</td>
<td>Assistant Professor of Materials Science</td>
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<td>EPFL (Switzerland)</td>
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<td>14:50</td>
<td>Coffee break and posters session</td>
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<td>15:20</td>
<td><strong>Prof. Simone Schürle, Ph.D</strong></td>
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<tr>
<td>15:20</td>
<td>Assistant Professor of Responsive Biomedical Systems</td>
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<tr>
<td>15:20</td>
<td>ETH Zurich (Switzerland)</td>
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<tr>
<td>16:00</td>
<td>Feedback and closing remarks &gt;&gt; <strong>Group photo #2</strong></td>
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<tr>
<td>19:00</td>
<td><strong>Farewell Dinner</strong></td>
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<td>Restaurant Belcanto (Located in the historic building of</td>
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<td>Zurich Opera House with views over Lake Zurich. Serves</td>
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<td>Zurich cuisine). Address: Falkenstrasse 1, 8008</td>
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KEYNOTE PRESENTATION

Dennis Discher | Matrix-Nucleus structural coupling in tissues & mimetics: From the first organ to anti-cancer macrophages

Scaling concepts have been successfully applied for many years to synthetic polymers, but applications to biology seem under-developed even though cells and tissues are built from polymers. Tissues such as brain and fat are very soft while tissues such as muscle and bone are stiff or even rigid – even when probed at the nanoscale, but the effects on cells are just now being uncovered. Having shown that matrix stiffness helps specify tissue lineages in vitro [1], we developed mass spectrometry algorithms to quantify protein levels in embryonic, mature, and cancerous tissues and also studied cells on substrates of tuned stiffness [2]. Extracellular collagen polymers directly determine tissue stiffness with near-classical scaling, and for embryonic heart, contractile beating of the organ and of isolated cells on synthetic gels is maximal when the stiffness is that of the normal tissue, consistent with a ‘use it or lose it’ mechanism of tension-inhibited degradation. Actomyosin assembly likewise increases with stiffness and stresses the nucleus, which upregulates a nuclear structure protein called lamin-A (related to keratin in fingernails) that again scales with stiffness via ‘use it or lose it’. Lamin-A assembly has evolved to control nuclear stiffness and strength, and it varies widely between tissues and diseases including cancer. Differentiation is generally modulated by lamin-A levels downstream of matrix stiffness, with various pathways co-regulated by lamin-A. Complementary insights are obtained for DNA damage and repair with stem cells and cancer cells [3], with evidence of invasion through rigid pores providing insight into mutation scaling in cancer.


KEYNOTE SPEAKER

Dennis Discher is the Robert D. Bent chaired Professor at the University of Pennsylvania and Director of a National Cancer Institute-funded Physical Sciences Oncology Center at Penn, where he has been since 1996. His lab discovered matrix elasticity effects on stem cell differentiation and generally takes a soft matter physics and polymer approach to cell biology questions. Recent efforts focus most specifically on mechanobiological determinants of DNA damage and genome variation, as well as macrophage engineering to infiltrate and attack tumours.
Theme I:
The Regenerative Matrix

< False-coloured cryogenic scanning electron micrograph (Cryo-SEM) of a single human stem cell (centre of image) embedded within a porous hydrogel matrix. The hydrogel is composed of thiolated hyaluronic acid cross-linked with poly(ethylene glycol) diacylate. Credits to Silvia A Ferreira, Cristina Lopo and Eileen Gentleman, KCL.
Modifiable hydrogels have revealed tremendous insight into how physical characteristics of cells' 3D environment drive stem cell lineage specification. However, in native tissues, cells do not passively receive signals from their niche. Instead, they actively probe and modify their pericellular space to suit their needs, yet the dynamics of cells' reciprocal interactions with their pericellular matrix when encapsulated within hydrogels remains relatively unexplored. We have recently shown that human bone marrow stromal cells (hMSC) encapsulated within hyaluronic acid-based hydrogels modify their pericellular environment through degradation and/or protein secretion, imparting them with similar pericellular stiffnesses, regardless of initial hydrogel properties. These cell-secreted pericellular matrices play a role in regulating hMSC fate, with secretion of a stiff proteinaceous pericellular matrix associated with adipogenesis, and degradation with osteogenesis. Our observations suggest that hMSC participate in a bi-directional interplay between the properties of their 3D milieu and their own secreted pericellular matrix, and that this combination of interactions drives fate.

Eileen Gentlemen is Senior Research Fellow and Principal Investigator at King’s College London. The Gentleman lab works at the interface of stem cell biology, chemistry and materials science to develop innovative biomaterials for regenerative medicine. Their aim is to understand how materials and their properties from the nano- to macro-scales affect stem cells. They then exploit these insights to develop 3D materials that can effectively direct stem cell differentiation for tissue engineering.
Boris Hinz | Matrix and Myofibroblast Mechanics in Fibrosis

Tissues lose integrity upon injury. To rapidly restore mechanical stability, a variety of different cell types are activated to acquire a reparative phenotype — the myofibroblast. Hallmarks of the myofibroblast are secretion of extracellular matrix (ECM), development of adhesion structures with the ECM, and formation of actomyosin contractile stress fibres. Rapid repair comes at the cost of tissue contracture due to the inability of the myofibroblast to regenerate tissue. When contracture and ECM remodelling become progressive and manifest as organ fibrosis, stiff scar tissue obstructs and ultimately destroys organ function. Pivotal for the formation of myofibroblasts are mechanical stimuli arising during tissue repair and chronic persistence of inflammatory cells. High stress, partly being a consequence of myofibroblast activities, amplifies scarring whereas absence of stress suppresses myofibroblast activities. I will give an overview on our current projects that address how mechanical factors orchestrate the development of myofibroblasts: (1) by mechano-sensing of tissue stiffness, (2) controlling the bioavailability of pro-fibrotic TGF-β1, and (3) mediating communication between myofibroblasts and macrophages. By understanding and manipulating myofibroblast and macrophage mechanoperception we will be able to devise better therapies to reduce scarring and support normal wound healing.


Boris Hinz is Distinguished Professor at the Laboratory of Tissue Repair and Regeneration of the Matrix Dynamics Group, Faculty of Dentistry, University of Toronto. His lab aims to understand the mechanisms controlling myofibroblast function and how they influence the development of fibrosis. They carry out functional analysis of the cell’s contractile apparatus (actin stress fibres), of force transmission at sites of cell-extracellular matrix contacts (focal adhesions) and of the mechanical cross-talk between contractile stress fibres of contacting fibroblasts at sites of cell-cell adherens junctions. They also develop novel strategies to counteract myofibroblast malfunction by targeting these instrumental structures of the contractile phenotype.
Jérôme Feige | Targeting the ECM of the Stem Cell Niche to Restore Muscle Regeneration in Aging

The remarkable ability of skeletal muscle to regenerate upon injury is conferred by tissue-resident stem cells called satellite cells. With age, the regenerative capacity of muscle stem cells (MuSCs) dramatically declines. Developing strategies to enhance muscle repair in elderly people is therefore required, in particular to accelerate their recovery from injuries following falls or from surgical interventions affecting muscle tissues. As the causes of MuSC dysfunction with age are multi-systemic, we have investigated age-related changes at different levels of the MuSC niche, in order to uncover synergistic ways to restore regenerative capacity in aged skeletal muscle. Our results demonstrate that interventions targeting the extracellular matrix, cell-cell interactions in the stem cell niche and circulating peptides can rescue skeletal muscle regenerative failure during aging by restoring youthful muscle stem cell function.


Jérôme Feige is Group Head at the Nestlé Institute of Health Science (NIHS), based in EPFL, Switzerland. The mission of his group is to study the mechanisms leading to the dysfunction of skeletal muscle during aging, particularly the condition of sarcopenia, in which muscle mass and muscle function decline due to age. His research approach focuses on understanding the different pathophysiological mechanisms of sarcopenia to provide novel concepts for intervention strategies and biomarker discovery.
Theme II:
The Mechano-Matrix

< Tendon fibres, coloured transmission electron micrograph (TEM).
After a decade of recognition that extracellular matrix (ECM) properties can influence cell behaviour to similar degree as growth factors, in particular ECM composition, topography, porosity, and elastic modulus (i.e. stiffness), biologists have come to recognize its importance. However matrix is highly dynamic, changing how much of it is secreted and assembled during development and disease. Most synthetic ECM mimics made initially to study this phenomenon were static but there is growing interest in making matrices that have tunable properties with time. Using several material systems in 2D and 3D, I will highlight how we have used matrix dynamics to study mechanisms in heart disease. I will employ the differentiated cardiac progeny of induced pluripotent stem cells (iPSCs) to understand how genomic variants that predispose progeny in an engineered niche to higher risk for coronary artery disease and myocardial infarction. While mechanisms in protein-coding loci are obvious, variants in non-coding loci or when multiple variants are present in combination are difficult to determine, and so I will use our materials-based approach to highlight methods that can induce disease-in-a-dish in order to better study mechanisms. Based on these exciting results, I will advocate that any in vitro culture system should employ 3D, dynamic materials or natural ECM that change as the niche does in vivo.


Adam Engler is Associate Professor of Bioengineering at the University of California, San Diego. The Engler lab is focused on the mechanobiology of cardiovascular diseases, cancer, and aging. They develop microfabricated technologies and biomaterials to examine how cell behaviour is directed by the extracellular matrix. In cases where this is more difficult, such as aging, they also use a host of model organisms like the fruit fly.
Emerging evidence suggests that primary and stem cells respond not only to current matrix properties but also store information about previous environmental cues. To investigate the hypothesis of cellular mechanical memory, we developed photoresponsive, poly(ethylene glycol) (PEG)-based hydrogels whose moduli can be modulated in the presence of cells with cytocompatible irradiation. O-Nitrobenzyl ether moieties were integrated into the backbone of PEG hydrogels to provide a photolabile handle for spatial and temporal control of substrate modulus with single or multiphoton light sources.[1] The platform, therefore, enables spatial patterning of gel mechanics in 2D and 3D in the presence of live cells. We exploited this platform to understand reversible activation of porcine valvular interstitial cells (VICs) and identified substrates that can maintain in a quiescent phenotype during culture.[2] In addition, the platform was used to investigate the hypothesis of mechanical memory in mesenchymal stem cells (MSCs).[3] This work illustrates the need to consider how we culture primary and stem cells outside of the body and that the past history of culture can influence future cell fate.


Mark Tibbitt is Assistant Professor of Macromolecular Engineering at ETH Zürich, within the Institute of Process Engineering of the Department of Mechanical and Process Engineering. His lab we developed user-tunable cell culture systems based on bio-orthogonal chemistries that afford spatiotemporal control over material properties with light. More generally, his research integrates concepts and techniques from chemical engineering, synthetic chemistry, materials science, and biology to design and assemble responsive biomaterials.
Introduction: Tendon tissue homeostasis heavily depends on appropriate mechanical loading within a narrow, but still poorly defined, physiological range. We will discuss our recent work trying to quantify the cell-matrix interactions that drive tendon tissue homeostasis and how these interactions regulate matrix remodelling in repair and/or eventual tissue degeneration. We will discuss the Snedeker Laboratory’s roadmap for unravelling these mechanically regulated signalling pathways, a roadmap designed to enable research and development toward effective treatment strategies for connective tissue disease.

Results, Discussion, and Perspective: Our recent studies suggest that tissue damage accumulates in the tendon until either mechano-sensitivity is lost and/or “intrinsic repair mechanisms” are overwhelmed. In the latter case, it is plausible that the metabolic cost of extracellular matrix remodelling exceeds the locally available nutrient supply. We hypothesize that upon reaching this “Metabolic Tipping Point”, the vascular system is recruited along with accompanying nerve supply (and pain). The tissue may then descend into a chronic disease state characterized by high matrix turnover and increasingly poor tissue quality. According to this paradigm, a delicate mechanically regulated balance exists between recruitment and suppression of the extrinsic vascular system by the resident tendon core cells – a handshaking across tissue compartments that potentially involves complex interplay with the innate immune system.


Selman Sakar | Micromechanics of Self-Organization in Fibrous Tissues

Cells primarily exist embedded within an information-rich three-dimensional microenvironment that contains multiple extracellular matrix components. The microscale blueprint of these fibrous networks constrains spatially where cells can form adhesions and imparts complex mechanical characteristics due to viscoelastic response to loading and anisotropy in structure. Deciphering the dynamic communication among cells connected through a complex fibre network requires a rigorous engineering analysis. High-throughput biomimetic microscale platforms provide control over initial geometry and composition of tissues in a chemically defined optically visible microreactor environment. Combined with force microscopy and genetic tools, quantification of changes from cytoskeleton to the bulk properties becomes feasible. In this lecture, we are going to explore robotic manipulation techniques that allow probing of the dynamics of multicellular interactions in their social context at the tissue as well as the cellular level. We will also discuss the powerful benefits of marrying computational models with experimental observations to investigate the physical mechanisms of self-organization and matrix remodelling.


Selman Sakar is Assistant Professor of Mechanical Engineering at EPFL, Switzerland, and head of the Microrobotic Systems Laboratory. His lab is developing advanced high-throughput technologies for investigating the complex architecture, function and regeneration of soft tissues and collective behaviour in groups of model organisms. To uncover fundamental principles of adaptive self-organization and physicochemical communication, he employs various methodologies including microrobotics, microfabricated tissue platforms, electromagnetic and optical manipulation systems, advanced microscopy, and synthetic biology.
Theme III:
The Immuno-Matrix

False-colour, Z-depth projection of human tendon-derived stromal cells encapsulated with a 3D synthetic Polyethylene glycol (PEG) hydrogel. Credits to Amro A. Hussien, Laboratory for Orthopedics Biomechanics, ETH Zurich & Uniklinik Balgrist, Switzerland.
Kim Midwood | Decoding immuno-modulatory signals from the extracellular matrix: new strategies for treating inflammatory diseases?

An immune response to pathogen invasion is triggered upon detection of pathogenic molecules by innate immune sensors, whilst non-infectious threat is signalled by endogenous inflammatory stimuli. Although insights into pathogen pattern recognition have revealed a great deal about the molecular basis of host defence against infection, it is not well understood how endogenous molecules created during tissue damage and cellular stress, or associated with tumorigenesis, activate immunity. Previous work revealed how extracellular matrix molecules specifically induced upon tissue injury create a pro-inflammatory niche that enables resident stromal cells and infiltrating immune cells to survive and thrive, and within which matrix molecules directly activate site-specific inflammatory programmes. Here I will discuss how matrix molecules are recognized as inflammatory triggers, the distinct immune signalling pathways they activate, and the importance of identifying pathologically relevant forms of matrix constituents to understanding their contribution to inflammation. I will also talk about how we are translating these discoveries into novel diagnostic tools, and developing new drugs that target the microenvironment to enable selective amelioration of aberrant ‘sterile’ inflammation in autoimmune and fibrotic diseases, and in tumours.


Andrea Ablasser | Intracellular DNA sensing in health and disease

The life of any organism depends on the ability of cells to accurately recognize and eliminate harmful microbes. In order to detect the immense repertoire of pathogenic entities, the innate immune system of mammals has evolved distinct sensing strategy, a major one of which is based on the recognition of DNA. Integral to this process are intracellular DNA-binding proteins that, upon interaction with DNA, initiate tightly regulated signalling cascades that trigger a series of cellular events, which ultimately promote an inflammatory response. While this process was originally discovered as crucial component of innate immune defence against pathogens, recent work has elucidated a role for cytosolic DNA recognition pathways beyond the traditional realm of innate immunity.

In this talk I will present an update of our current research progress on innate DNA sensing through the cGAS-STING pathway - a pivotal cytosolic DNA sensing system. In particular, my talk will focus on discussing the role of cGAS in the recognition of chromatin herniation in the context of cellular senescence and laminopathies. I will also present more recent data related to the pharmacological targeting of the cGAS-STING pathway in autoinflammatory disease.


Andrea Ablasser is Assistant Professor of Life Sciences and Principal Investigator at EPFL, Switzerland. The goals of her research is to understand how the cells of the immune system detect the presence of pathogens and to dissect the fundamental mechanisms that provide host defence. Her lab focuses on the identification of factors involved in the intracellular recognition of pathogen-derived molecular patterns and on the elucidation of the consecutive signalling events. She also aims towards a better understanding of the physiological functions of these pathways both in the context of pathogen infection and during non-infectious, physiological processes.
Leukocyte migration through afferent lymphatic vessels

Afferent lymphatic vessels mediate the transport of soluble antigen and leukocytes to draining lymph nodes, thereby serving as immunologic communication highways between peripheral tissues and secondary lymphoid organs. The main cell types migrating via this route are antigen-presenting dendritic cells (DCs) and antigen-experienced T cells. While DC migration is important for maintenance of tolerance and for induction of protective immunity, T cell migration through afferent lymphatics contributes to immunosurveillance. In contrast to migration through blood vessels, the detailed molecular and cellular requirements of leukocyte trafficking through afferent lymphatics have only recently started to be unravelled. Particularly time-lapse confocal- or 2-photon-based imaging, performed in skin explants or in anesthetized mice, has recently allowed to better study this migratory process at the single-cell level. These studies have revealed that leukocyte trafficking through afferent lymphatic vessels follows a step-wise migration pattern, involving chemokine-mediated migration towards lymphatic vessels, transmigration into the vessel lumen, active migration within lymphatic capillaries followed by passive transport from the downstream contracting collecting vessels towards the draining lymph node. In my presentation I will summarize and discuss current knowledge of leukocyte migration through afferent lymphatic vessels and present selected examples from our research, which employs intravital microscopy and other techniques to further elucidate this migratory process.


One of the most recent Review Articles on the topic


One of our recent Research Articles on the topic with links to intravital microscopy movies

Cornelia Halin Winter is Associate Professor of Pharmaceutical Immunology at ETH, Zurich. Her research focuses on how leukocytes transmigrate through lymphatic endothelium. Leukocyte migration into lymphatic vessels represents one aspect of leukocyte trafficking, which has only been marginally studied to date. An important goal of her research is to identify and validate new (adhesion) molecules, which could serve as therapeutic targets to modulate immune responses.
Enhancing cancer immunotherapy using responsive biomaterials

Adoptive cell therapy (ACT) employing antigen-specific T-cells has elicited dramatic clinical responses in leukaemia and a subset of melanoma patients. However, strategies to safely and effectively augment T-cell infiltration and function in solid tumours remain of great interest. Our laboratory aims to enhance adoptive T-cell therapy and other cancer immunotherapies through responsive nanoparticle drug delivery. Here we describe a strategy to enhance the tumour-infiltration and function of transferred T-cells by spatiotemporally controlled delivery of immunomodulators. Responsive protein nanogels (NGs) containing large quantities of immunomodulatory drugs are designed and synthesized to release the drugs in response to the reductive environment specific in tumour tissue or on T-cell surface. We show that T-cells increase their cell surface reduction potential upon activation, which we exploit through the design of cell surface-bound NGs that disassemble to release protein cargos in response to this change in the local reductive environment following T-cell receptor (TCR) triggering. The T-cell surface-bound NGs selectively release adjuvant drugs in response to TCR activation, focusing drug release in sites of antigen encounter such as the tumour microenvironment. Using an IL-15 superagonist complex as a candidate adjuvant drug cargo, we demonstrate that relative to systemic administration of free cytokines, NG delivery selectively expands adoptively transferred T-cells 16-fold in tumours, and allows at least 8-fold higher doses of cytokine to be administered without toxicity, leading to substantially increased anti-tumour efficacy and safety. This strategy provides a general approach to augment the function of cell therapies by linking drug release to cell function in vivo.


Li Tang is Assistant Professor of Material Sciences at EPFL, Switzerland, and head of the Laboratory of Biomaterials for Immunoengineering. The overarching goal of the Tang laboratory is to establish detailed understanding of the interactions between synthetic biomaterials and the immune system, and precisely control the immune response using engineered intelligent biomaterials in order to develop effective novel immunotherapies for cancer, infectious diseases and autoimmune disorders. His lab leverages the power of state-of-the-art synthetic chemistry, materials science and nanotechnology to advance the diagnosis and therapy of human diseases by modulating the immune system with smart biomaterials.
Theme IV:
The Responsive Matrix

< Mouse intestinal organoid, growing and budding within a synthetic hydrogel of functionalised Polyethylene glycol (PEG). Nuclei stained in blue, Actin stained in green, and LFAB2, marking differentiated enterocytes, stained in magenta. Credits to Saba Rezakhani, Laboratory of Stem Cell Bioengineering, EPFL, Switzerland.
In this talk I will describe cutting-edge technologies for engineering functional tissues, focusing on the design of new biomaterials mimicking the natural microenvironment, or releasing biofactors to promote stem cell recruitment and tissue protection. In addition, I will discuss the development of patient-specific materials and 3D-printing of personalized vascularized tissues. Finally, I will show a new direction in tissue engineering, where, micro and nanoelectronics are integrated within engineered tissues to form cyborg tissues. In this new concept the built-in electronic network is used to on-line record cellular electrical activity and when needed to provide electrical stimulation for synchronizing cell contraction. Furthermore, electroactive polymers containing biological factors can be deposited on designated electrodes to release drugs in the cellular microenvironment on demand, affecting the engineered tissue or the host.


Tal Dvir is Associate Professor of Biotechnology, Materials Science and Engineering at Tel Aviv University, Israel. Research interests of his lab include microfluidics-based tissue engineering, cardiac and neural tissue engineering, fabrication of nanoelectronics engineered tissue hybrids, and development of smart delivery systems that recruit stem cells to defected organs.
Maartje Bastings | Programming materials with cell-adhesion sites that are functionally indistinguishable from nature

Understanding mechanisms and consequences of receptor co-localization and inter-receptor communication is critical for the design and development of therapeutic particles and functional biomaterial scaffolds. Integrins are transmembrane cell receptors with a dual role in both cell adhesion and cell-signalling pathways. Fibronectin (FN) is one of the protein polymers present in the extracellular matrix (ECM) involved in cell-adhesion. FN contains multiple binding clusters that can interact with 12 different types of integrins. The RGD peptide recognition motif found in FN binds to α5β1 integrins with an affinity of ~10 μM and has been extensively used to add cell-adhesion functionality to synthetic materials, yet FN has more to offer than just RGD. PHSRN, a peptide sequence derived from a neighbouring FN domain, binds synergistically to the α5β1 integrin, yielding an overall 100-fold stronger binding. The RGD and PHSRN peptides are found 35Å apart in FN. Two peptides, LDV and REDV, are found ~17nm downstream from the RGD/PHSRN binding cluster on FN and bind the α4β1 integrin.

Our experimental goal is to design materials that present these peptides with similar spacing and orientation as found in FN. We hypothesize that through spatial control in heterogeneous ligand presentation, our materials will interact with both integrins on the cell surface in a fashion that is functionally indistinguishable from the natural FN-cell interaction. We use DNA nanotechnology to create material scaffolds of defined size and shape. Biofunctionality is included via sequence-guided self-assembly between the material backbone and DNA-tagged FN mimicking peptides.


Maartje Bastings is Assistant Professor of Materials Sciences and head of the Programmable Biomaterials Laboratory at EPFL, Switzerland. Research in her lab focuses on the development of programmable guidelines to control self-assembly of supramolecular materials. Through the addition of DNA, the ultimate programmable biopolymer, at the cell-material interface, she aims to transfer information provided by the cell-surface to guide the assembly of polymers and peptides into the perfect ECM mimicking environment. Her lab combines expertise in polymer chemistry, DNA and peptide synthesis and protein engineering with bioconjugation techniques to create novel building blocks for nanoparticle and biomaterial self-assembly.
Simone Schürle | Mehanosignaling and enhanced mass transport in tumor ECM models using micro-robotics

The tumour is host to a complex, dynamic microenvironment that arises from interactions of malignant and non-transformed cells. Understanding the underlying factors driving initiation, growth, and expansion is key to designing appropriate diagnostic and therapeutic tools, which then need to be delivered effectively.\(^1,2\) In my lab, we aim to shed light on mechanical cues from the ECM that drive tumour proliferation and develop mechanisms to adequately shuttle nanodrugs across the ECM for cancer therapy. Recent findings on strain-enhanced stress relaxation of cancer cells suggest that cyclic strain loading induces cellular relaxation and interrupts the stiffening cascade, thereby also disrupting the mechanical cues for growth and expansion.\(^3\) We work with tumour mimicking microfluidic models and study cellular responses to controlled cyclic mechanical stimuli applied via a magnetic manipulation platform. By dispersing magnetic microparticles within collagen-cell matrices, mechanical torques can be applied though external magnetic fields using our microrobotic platform. Cyclic mechanical stresses can be introduced to study changes in cellular expression levels and simultaneously monitor strain to deduce stiffness changes in the collagen matrix. With this approach, we aim to identify mechanisms in the metastatic cell signalling cascade. Our model further enables us to investigate strategies to enhance drug accumulation and penetration in the dense ECM of the tumour microenvironment. For this, we employ local convection enhancement initiated by magnetic micropropellers either near or within collagen matrices that locally and wirelessly drive convective transport of nanomedicine.


Simone Schürle is Assistant Professor of Responsive Biomedical Systems at ETH Zurich. Her lab develops diagnostic and therapeutic systems at the nano-and microscale with to tackle a range of challenging problems in health care, including the increasing cancer burden and infectious diseases. Her group develops tools to study disease mechanisms in vitro at the cellular level, and then uses this knowledge to inform the design and fabrication of responsive nanosystems that help diagnose or treat diseases with minimal invasion.
Abstracts from participants

< Rotary shadow electron microscopy of purified laminin protein. Alpha, beta, and gamma chains can be seen defining the three short arms at one end of the protein. At the other end, the long arm ends with integrin-binding globular domains.
In the lately, tumour-associated macrophages (TAM) were identified as important contributors for tumour malignancy[1]. Macrophages are highly plastic cells with a wide spectrum of phenotypes. Two main phenotypes have been described: pro-inflammatory and tumour resistant (M1), and matrix remodelling and prohealing (M2) macrophages which in a tumour environment promote malignancy. TAM have a typical M2 phenotype, creating an environment favourable for tumour cells. Better understanding of the different phenotypes could enable early detection of risk factors[1] or/and development of novel therapy mechanism[2]. For both groups, a specific set of biochemical cues were identified[3]. In addition, the role of the extracellular matrix’ (ECM) mechanical properties on macrophage plasticity was assessed[4]. Stiffer ECM was found to promote M2 macrophages, a microenvironment widespread within tumours. The aim of this research is to understand how the substrate stiffness affects the biomechanical forces that M2 macrophages apply to their microenvironment, e.g. during phagocytosis. For this aim, a 5 degree of freedom magnetic tweezer system[5] is used in combination with magnetic microparticles that are covalently decorated with antibodies. These “micropreys” are utilized in forceclamp measurements between the immune cells and the particles[6]. The macrophages are cultivated on glass and silicone-based elastomers of defined stiffness ranging from 0.4 – 70 kPa[7]. The substrates are covalently functionalized with fibronectin to allow for integrin-based cell attachment.

5. S. Schuerle, M. S. Sakar, A. Meo, J. Moeller, B. E. Kratochvil, C. S. Chen, V. Vogel, B. J. Nelson,
In the human body, tissues and organs provide complex mechanical environments in which reciprocal interactions between cells and the extracellular matrix (ECM) are constantly taking place and regulating several physiological processes. Identifying and understanding the environment created by the cells is hence a crucial step towards the rational design of new ECM-inspired materials for tissue engineering and regenerative medicine, as well as for the identification of the decisive factors involving processes like wound healing, ageing or disease.1,2,3

Among the various well-established three-dimensional cell culture platforms, synthetic hydrogels are widely used due to their biocompatibility, absence of inherent chemical cues and design flexibility. A modular PEG-based hydrogel containing both cell adhesive and proteolytic sites was developed by Ehrbar et al.,4 and has extensively been used for cell culture.5-8 The system is highly tunable in terms of stiffness and composition, and relies on a MMP-sensitive sequence for hydrogel degradation. Taking this material as a starting point, we have recently designed a new type of highly specifically cleavable hydrogel that allows the isolation of cells and cell-secreted ECM components without compromising their viability and integrity, respectively. We hypothesize that these innovative hydrogels will enable the downstream analysis of retrieved cells and ECM with comparable / identical methods as they have been established for 2D cultures.9,10

The translation of safe and effective drugs into products is currently restricted by the lack of control and low scalability of drug delivery systems. An additional problem is the high cost of drug development, which is heavily influenced by the complexity, limits and ethics of animal models. In this perspective, the controlled synthesis of drug delivery vehicles and the development of reliable drug-testing platforms are of central importance.

Drug-loaded polymeric nanoparticles (NPs) are an important class of drug delivery vehicles that can be fabricated via nanoprecipitation by mixing an organic precursor solution containing drug and polymer with water. The size of the NPs is a key parameter that influences both biodistribution and cellular uptake [1]. Further, clinical utility requires scalable and reproducible fabrication of NPs. These criteria have been met in microfluidic mixing devices, which have emerged for the controlled assembly of drug-loaded NPs [2]. In our work, we engineered a coaxial jet microfluidic mixing device to synthesize NPs from poly(ethylene glycol)-b-poly(lactide) (PEG-b-PLA) block copolymers and their size was tuned reproducibly between 40 and 200 nm. Preliminary encapsulation of drug-mimetic compounds (i.e., retinyl acetate) demonstrated that our system is suitable for the synthesis of drug-loaded NPs.

The next step of the project involves the study of the therapeutic efficacy of drugs by utilizing in vitro drug-testing platforms. Literature shows that organ-on-a-chip models and organoids are able to recapitulate specific properties of native tissues thus making them suitable for the simplification of complex organ functions to a small number of physiological processes. These platforms are envisioned to be less complex compared to animal models, closer to human conditions and less ethically problematic [3].

We envision that the combination of the controlled synthesis of drug-loaded NPs with reliable in vitro drug testing platforms will make drug formulations more accessible and efficient.

Elena Cambria | TRPV4 in stretch-induced inflammation of invertebral disc cells

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Mechanical loading and inflammation interact to induce disc degeneration and pain. Cyclic stretching influences the expression of anabolic, catabolic and inflammatory mediators. The transient receptor potential vanilloid 4 (TRPV4) channel was shown to regulate stretch-induced inflammation in mouse lung epithelial cells. However, its role in human discs is unexplored.

Primary human annulus fibrosus cells (n=2-3 donors) were seeded on fibronectin-coated PDMS chambers and cyclically stretched for different durations (1, 2, 4, 8 or 12 hours) at 20% strain and 1 Hz frequency on a commercial bioreactor. In TRPV4 inhibition experiments (n=4), cells were stretched for 1 hour in presence or absence of the specific TRPV4 antagonist GSK2193874 at different concentrations. Gene expression was analyzed by RT-qPCR. Activation of MAP kinases (p-38, JNK, ERK) was tested after 15 min stretching (n=2).

Gene expression of IL6, IL8 and COX2 was significantly upregulated by 1 hour stretching compared to controls, and decreased with longer durations. TRPV4, MMP1, MMP3, COL1A1, COL2A1 and ACAN expression was unaltered. Stretch-induced upregulation of IL6 and COX2 was reduced by GSK2193874. After 15 min of stretching, higher phosphorylation of p-38, JNK and ERK was observed compared to controls, and was reduced by GSK2193874.

We show that upregulation of inflammatory genes after 1 hour cyclic stretching and MAPK activation are reduced by inhibition of TRPV4. TRPV4 may thus constitute a potential therapeutic target to tackle disc degeneration and pain. Current work includes analysis of the conditioned medium by ELISA, and real-time calcium flux imaging during stretching to confirm TRPV4 activation.

Giacomo Chizzola | Quantifying Complex Multivalency: DNA Dendrons as Programmable Multivalent Nanoparticles

Programmable Biomaterials Laboratory PBL - EPFL

Multivalent interactions in nature are abundant and are relevant to many biological phenomena such as signal transduction, immune responses and cell-cell interactions. (2) Mastering and implementing these mechanisms in nanoparticles may lead to effective targeting and greatly increased selectivity in diagnostic and therapeutic applications. Control over the assembly of nanostructures is essential to achieve this goal. Self-assembly is a promising approach, but precise information and instruction needs to be encoded for the desired architecture to arise in high yields and as monodisperse as possible. Currently employed synthetic biomaterials lack the necessary spatial and heterogeneity control in ligand presentation.

DNA nanotechnology provides a perfect programmable material platform. Thanks to its predictable nucleotide complementarity, geometrically defined nanostructures can be formed by self-assembly of smartly designed DNA strands. Furthermore, DNA nanostructures...
can be functionalized with (sub-)nanometer precision to include bio-functionality for targeting, diagnostics and therapeutics (4). We can thus synthesize nanoparticles with heterogeneous populations of bioactive cues and investigate the effect of spatial control of ligand presentation.

Here, we start with a proof of concept where fully programmable dendritic nanoparticles interact with well-defined multivalent targets. Next, we will present heterogeneous ligands and target complex multivalency to mechanistically study the synergy in multivalent binding of RGD and PHSRN to the cell surface. The system will then be expanded to program more ligands and understand the role of spatial control in cell binding and signaling. This information can be translated into improved design of biomaterials and superior diagnostic nanoparticles.


Anna D’Agostino
SEMM-European School for Molecular Medicine, Naples, Italy

Osteogenic differentiation is a complex and still poorly understood biological process regulated by intrinsic cellular signals and extrinsic micro-environmental cues. Following appropriate stimuli, mesenchymal stem cells (MSCs) differentiate into osteoblasts through a tightly regulated multi-step process driven by several transcription factors and characterized by the expression of a number of bone-specific proteins. Here, we describe a novel transcription factor that we named Osteoblast Inducer (Obl)-1, involved in MSC differentiation towards the osteogenic lineage. Obl-1 encodes for a nuclear protein subjected to proteasomal degradation and expressed during osteoblast differentiation both in a murine multipotent mesenchymal cell line (W20-17) and in primary murine MSCs. RNAi-mediated knockdown of Obl-1 expression significantly impairs osteoblast differentiation and matrix mineralization with reduced expression of the osteogenic markers Runx2 and osteopontin. Conversely, Obl-1 over-expression enhances osteogenic differentiation and bone-specific markers expression. Obl-1 stimulates bone-morphogenetic protein (BMP)-4 expression and the consequent activation of the Smad pathway; treatment with a BMP receptor-type I antagonist completely abolishes Obl-1-mediated stimulation of osteogenic differentiation. Collectively, our findings suggest that Obl-1 modulates osteogenic differentiation, at least in part, through the BMP signalling pathway, increasing Runx2 activation and leading to osteoblast commitment and maturation.
Oksana Dudaryeva | SingleCellECM: Tunable 3D niches to study cell-ECM interactions on a single cell level

Oksana Dudaryeva, Mark Tibbitt
Macromolecular Engineering Lab, D-MAVT, ETH Zurich

Cell fate and function are regulated by biophysical and biochemical signals provided by their local microenvironment. These signals include chemical identity of the extracellular matrix (ECM) proteins as well as niche stiffness, topography and geometry. [1] The specific and synergetic effects of these cues on cell behavior, however, are still not fully understood. The main reason for this lack of understanding is the difficulty of controlling the individual cellular microenvironments in a complex 3D setting in conventional bulk encapsulation platforms. [2]

To address this, new types of single cell encapsulation platforms are emerging in the field of biomaterials. [3] The SingleCellECM is a novel type of cell culture platform that provides a high degree of control over the properties of single cell microenvironments. It consists of geometrically defined microniche arrays patterned on bioactive and photoresponsive hydrogel substrates. These niches can be occupied by single cells and their volume, geometry and mechanical properties are tunable allowing us to study the effects of geometrical cues, initial mechanical properties and ligand concentration on cell behavior with single cell resolution. Preliminary results show that MSCs encapsulated within the microniches show oriented spreading and cytoskeletal arrangement according to the distinct geometrical cues and substrate stiffness.


Amro A. Hussien | Elevated matrix tension derives a myofibroblastic phenotype in tissue-engineered tendons

Amro A. Hussien(1, 2), Robert Knell(1), Jasper Foolen(3), Jess G. Snedeker(1, 2)

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Tendinopathies are characterized by dysregulated fibro-inflammatory healing processes that ultimately result in tissue scarring and loss of function [Goodier HC, 2016]. Along with soluble molecules, biophysical cues (e.g. ECM stiffness, mechanical forces) may be key drivers of aberrant myofibroblast activation; a hallmark of fibrosis. However, a quantitative understanding of how 3D ECM mechanics contribute to pathological healing is hindered by relatively inaccessible experimental approaches [Legant, 2009; Polacheck, 2016]. To address this challenge, we have developed a modular, cantilever-based platform that allows culture of 3D tendon-like constructs under easily variable static tension. The system also allows an online readout of tissue tension and cell-
generated forces. We exploited the system to demonstrate that high levels of tissue tension strongly influence tendon-derived stromal cells, with cells exhibiting a 25-fold increase in cell-generated traction forces compared to soft flexible controls. Additionally, a tendency towards a (proto-)myofibroblastic differentiation was observed, with a significant downregulation of key tendon-related transcripts (scleraxis, tenomodulin and mohawk), and an increased expression of α-smooth muscle actin at the protein level. Further ongoing work is focused on systematically dissecting what role ECM composition and mechanical tension play in informing tendon cell-ECM interactions towards a fibro-inflammatory activation and/or homeostatic remodelling.


Lisa Krattiger | Microfluidic in vitro model of the microvasculature for the study of circulating cancer cell extravasation

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In metastasizing cancer, malignant cells invading and intravasating into the lymphatics or the blood system may disseminate to distant organs such as bone or the lung where they exit the circulation and move into the parenchyma through the process of extravasation. While much research has been done investigating the role of soluble factors on extravasation, a potential contribution of the endothelial glyocalyx and the local extracellular matrix (ECM) have not been studied in depth.

The overall aim of this project is to develop a perfused in vitro model that closely mimics the microvasculature and angiogenic processes observed in vivo. Established microfluidic tools, biofabrication techniques and co-culture systems will be employed, combined and refined in order to reach this goal. More specifically, human umbilical vein endothelial cells (HUVECs) will be cultured alongside other cell types (e.g. bone marrow-derived mesenchymal stem cells [BM-MSCs]) within biocompatible hydrogel materials. These cell-material constructs will be embedded into a microfluidic device which allows for controlled perfusion of the constructs. Design of the microfluidic device, composition of the cell-hydrogel constructs, as well as the hydrogel material itself will be optimised. The optimised model system will be validated against common in vivo models. The in vitro model will then be further improved to allow for increased throughput analyses and finally applied to elucidate the role of extracellular factors, such as the presence and composition of the endothelial glyocalyx, ECM, as well as flow dynamics on circulating cancer cell extravasation.
Integrins are transmembrane receptors that mediate cell adhesion and are vital in tissue engineering. To date 24 different integrin heterodimers have been identified to respectively distinguish extracellular matrix (ECM) recognition epitopes via multivalent and dynamic receptor clustering. [1] Material interfaces have been made bioadhesive through display of the RGD peptide sequence found on fibronectin (FN), a polymer of the ECM. A minimum spacing of 58 nm [2] has been found critical to ensure a functional cell-material interaction. Integrins are upregulated in diseased cells including many cancers [3], thus insights in their dynamic spatial organization is crucial in developing targeted therapeutic materials. Current material interfaces fail to perform a quantitative biospecific in vitro analysis of the spatial parameters in material-integrin signalling. This is caused by inherent polydispersity that arises from lack of control in synthesis at the nanoscale, resulting in a distribution in the presentation of ligands. Therefore, a programmable, modular system with nanometre precision is vital. DNA based nanotechnology afford the nanoscale control paramount in elucidating the role of spatially controlled ligand presentation in integrin signalling. Capitalizing on the nanoscale control of ligand presentation afforded by the DNA nanotechnology platform, we develop material interfaces with functionality that are indistinguishable from nature. When our ECM-mimicking nanostructures are integrated in hydrogels, we anticipate control over the self-assembly of dynamic materials that display unique super-selectivity toward cell adhesion.

increases the number of surgical interventions and poses an unnecessary burden on the patient. Injecting mesenchymal stem cells within a hydrogel that allows for their differentiation towards cartilage represents a promising alternative. Although, a balance must be found between the biomaterial's mechanical properties that should withstand the joint loads and its softness that allows for cell encapsulation and cell viability. Therefore, a highly tunable construct is needed.

**Material and methods:** In an enzymatically crosslinked tyramine-modified hyaluronic acid hydrogel (HA-Tyr), the viscoelastic properties were tuned by varying the concentration of the coupling reagents and the cell density. The cytocompatibility of the hydrogel was assessed by Live/Dead staining while its biomechanical properties were investigated by rheology and unconfined compression test. Subsequently, the cell-laden HA-Tyr hydrogels were exposed to multi-axial loading in an in vitro bioreactor that mimics in vivo joint loads. After 28 days, markers associated with cartilage formation were assessed.

**Results:** Sufficient biomechanical properties were ensured with high-density cell encapsulation and high cell viability was achieved after a period of 28 days. Multiaxial loading stimulated the production and the activation of transforming growth factor beta 1, a potent inducer of cartilage formation.

**Conclusion:** We confirmed cartilage formation in a HA-Tyr hydrogel after improving its mechanoresilience to enable the application of joint-mimicking loads. The possibility to expose a previously soft cell-laden biomaterial to mechanical loads lends hope for future implementation in cartilage repair strategies.

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Fatemeh Navaee | **Impact of the dECM-fibrin hydrogel on cardiomyocytes differentiation in co-culture with fibroblasts**

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3D cell culturing is important to mimic the structural and functional properties of native myocardium. There are several biomaterials that can be used in cardiac tissue engineering. However, major problems with current cardiac tissue engineered biomaterials include the risks of toxicity, immunogenicity, lack of compatible degradation paths, and inappropriate mechanical properties. To provide an appropriate hydrogel for 3D cardiac cell culture, we propose decellularized extracellular matrix (dECM) mixed with fibrin.

To demonstrate the impact of dECM-fibrin hydrogel on H9c2 cells differentiation, the pig heart has been decellularized and mixed with fibrin. By measuring the content of DNA, Collagen, Elastin, and GAGs, it proves that the hydrogel has essential components of ECM. The mechanical properties show its similarity to native tissue. The rheological properties investigate that its gelation time provides enough time to handle the cells in pre-gel. Then, a co-culture of H9c2 and fibroblast cells were cultured in the hydrogel.

The results show that this hydrogel promotes the attachment, spreading, and elongation of cardiomyocytes (CMs) due to its intrinsic biological properties and similarity to the mechanical properties of natural myocardium in its 3D structure. Also, using this hydrogel, we can eliminate the use of retinoic acid which is the main component for H9c2
differentiation. Therefore, we claim that it promote the differentiation and functionality of cardiac cells compare to other hydrogels. In conclusion, dECM-fibrin hydrogel is applicable in cardiac tissue engineering applications.

Vasileios Papalazarous | Effects of matrix stiffness on metabolism and ATP homeostasis of pancreatic cancer cells during invasion and migration.

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Extracellular matrix (ECM) rigidity is currently emerging as a major hallmark of tumorigenesis, where extensive ECM remodelling progressively increases the stiffness of the tumour microenvironment. This increase of ECM stiffness can act through mechano-signalling networks to promote cell invasion and migration, favouring disease progression and dissemination. Pancreatic cancer is a highly metastatic malignancy, usually presented with a stiff desmoplastic ECM. Tumours are characterised by limited oxygen and nutrient availability, posing major metabolic challenges to cancer cells. In contrast, tumours show increased energy requirements during cancer progression. Therefore, it is important to understand how cancer cells regulate ATP homeostasis to manage these demands. We find that pancreatic cancer cells respond to ECM stiffness in vitro both biologically by altering their shape and YAP-mediated mechano-signalling and metabolically by switching from aerobic glycolysis on a soft ECM to oxidative phosphorylation on a stiff ECM. This is accompanied by increased ATP turnover levels in response to high ECM stiffness. We discovered that the creatine pathway, an important circuit for ATP maintenance and recycling, is harnessed during mechanosensing upon a stiff ECM. Specifically, increased ECM stiffness promotes the expression of Creatine Kinase Brain (CKB), the critical enzyme for creatine-dependent ATP availability on cytoplasmic sites of high energy demand. CKB upregulation is accompanied by assembly of functional focal complexes and consequent YAP translocation to the nucleus. Targeting the creatine circuit hinders the migration of pancreatic cancer cells, indicating that the ATP pool generated by the creatine pathway could contribute to the invasive behaviour of pancreatic cancer cells. Therefore, we suggest a novel connection between biomechanical events and cell metabolism that could elucidate how the cells address their energetic challenges under excessive ECM remodelling.
Metastasis is the major cause of mortality in all cancer patients, but it still remains incurable. It is a multistep process which is greatly influenced by microenvironment, including modulation of the extracellular matrix (ECM) composition. It was known for long time that tumours originating from different sites of the body have different metastatic potential. Nonetheless, biology of this phenomena is not well understood and determining its molecular regulation will provide new therapeutic opportunities. Our laboratory studied the influence of site of tumour growth on melanoma metastasis in mice and found that ECM applied from the most aggressive to the least aggressive site was able to drive metastasis. Molecular profiling of the melanoma cells from these two sites revealed differences not at the genomic level but at the proteomic-phosphoproteomic signalling level. In my project I further study if particular epigenetic mechanisms are linked to the aggressive metastatic potential and if they are affected by the ECM composition. These findings will be additionally evaluated in the human patient samples and will provide a novel insight into how site of tumour growth influences cancer progression.

Saba Rezakhani | Chemically defined hydrogels for epithelial organoid culture

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Over the past few years, there has been a substantial effort to substitute ill-defined and animal-derived Matrigel as the main matrix for intestinal stem cell and organoid culture. Chemically-crosslinked hydrogels with tunable mechanical properties and presentation of adhesive ligands have potential to substitute Matrigel. However, when formed at low solid content favouring biological applications such as organoid culture, these hydrogels swell very extensively and lack mechanical integrity. These limitations arise from network defects such as primary loops that are formed during cross-linking. Here we report the synthesis of hybrid hydrogels by Michael-type addition reaction between vinyl sulfone- and acrylate-terminated multiarm PEG macromers with thiol-containing PEG precursors. These networks were formed at lower solid content with higher bioactive molecule concentration without losing mechanical integrity. We showed that these matrices were optimal for intestinal stem cell proliferation and subsequent differentiation. The resulting hydrogels provided a platform for the intestinal organoid formation and can be further optimized for expanding other types of stem cells and organoids.
The anchoring of cells to the ECM is an important function of integrins. Yet, these cell surface receptors also regulate cell survival, proliferation, migration and differentiation. Focal adhesions (FAs) are a striking example of integrin-based combinatorial signalling platforms, which integrate diverse environmental signals and subsequently modulate the cell behaviour. Paxillin, a FA-associated adaptor protein, is an essential hub in the assembly of FAs and in coordinating downstream signalling pathways, such as cell spreading, migration and proliferation (both occurring in physiological and pathological processes). The precise mechanism of paxillin recruitment to FAs, its regulation and feedback control of these structures, are still far from being fully understood.

Several binding partners of paxillin are known, though the identity of its recruitment sites in integrin-dependent adhesions have so far remained elusive. Deletion mutagenesis has previously found LIM3 domain as the principal determinant of paxillin FA localization. Our dynamics studies, based on photoactivation of paxillin within focal adhesions, are generating new data concerning the multifunctional adaptor protein paxillin. We now have evidence that (1) LIM domain deletions are not sufficient to determine the major anchoring domain, since the removal of a LIM domain compromises the 3D arrangement of the others, preventing their interactions with the corresponding binding partners. (2) Although extensive similarities in terms of structure, LIM domain are not interchangeable, but they rather have peculiarities previously unappreciated. (3) The highly positive-charged LIM4 domain contains two palmitoylation sites potentially involved in membrane binding and contributes to paxillin stability in FAs. Finally, (4) irreversible paxillin lipidation impacts integrins turnover. Our results will lead to a better molecular understanding of the paxillin domains that are required for integrin-dependent adhesion signalling and possibly provide the basis for modulation and interference with the downstream (paxillin-mediated) cellular responses.

Much is known about how biological cues direct early mouse development, generally in terms of the instructive role played by biochemical signalling gradients to pattern embryonic tissues to define the main axes of development and to allocate cells to distinct fates. Yet, processes such as gastrulation and early tissue morphogenesis also involve a complex geometrical restructuring of the embryo through coordinated cellular migration and extensive remodelling of local extracellular basement membranes, all within the spatial constraints of the uterine endometrium. In the light of such a complex tissue choreography and environmental constraints, it is becoming increasingly clear that biomechanical considerations might not be out of place. Indeed, it has been found that the mechanics (e.g.
stiffness, pressure) of the extraembryonic environment or of the embryo proper directly underpin key developmental events such as e.g. antero-posterior axis establishment or notochord emergence.

To better assess such putative biomechanical contributions, I use aggregates of mouse Embryonic Stem Cells as in vitro models of early development (Gastruloids). These embryonic organoids show spontaneous symmetry breaking and establish body axes and patterns of gene expression comparable to their in vivo counterpart. By interfacing such an experimental system with synthetic hydrogels engineered to display a range of stiffnesses, degradation rates, or even specific components of the in vivo basement membranes, biomechanical influences on embryonic patterning and morphogenesis can be studied in a controlled, precise, and high-throughput fashion. The observations stemming from the application of such a bioengineering approach to deconstructed embryonic models can be then related back to in vivo development and possibly open new investigative perspectives.
Social Event Programme:
Thursday 13 September
1. Meet at **9:30AM** at Bürkliplatz, Zürich

2. Take the boat at 9:40AM from Bürkliplatz to Küsnacht (Costs: 6.80.-CHF)

3. **10:20AM**: Start the beautiful hike through the Küsnacht Tobel towards Forch
   - 7.7 km
   - Duration: 2.5h – 3h

4. **1:30PM** arrive at Forch

5. **2:00PM** Take S18 back from Forch to Stadelhofen (Costs: 8.80.-CHF)
More information at www.ecmatrix.epfl.ch

Organizers:
Amro A. Hussien (ETH Zurich), Saba Rezakhani (EPFL), Angelina Schönenberger (ETH Zurich), Stefano Vianello (EPFL)
Supporting professors: Jess G. Snederker (The University & ETH Zurich), Selman Sakar (EPFL)