Nanobiotechnology for Cell Interfaces

733. WE-Heraeus-Seminar

17 - 18 March 2021
ONLINE

WILHELM UND ELSE HERAEUS-STIFTUNG

Introduction

The Wilhelm und Else Heraeus-Stiftung is a private foundation that supports research and education in science with an emphasis on physics. It is recognized as Germany's most important private institution funding physics. Some of the activities of the foundation are carried out in close cooperation with the German Physical Society (Deutsche Physikalische Gesellschaft). For detailed information see https://www.we-heraeus-stiftung.de

Aims and scope of the 733. WE-Heraeus-Seminar:

Recent years have seen exciting advances in engineering on the micro- and nanoscale to create surfaces that simulate specific physico-chemical features from natural cellular microenvironments. Lithography, DNA nanotechnology and other nano-enabled techniques have been exploited for probing and manipulating cells at the micro- and nanometer scale - the scale at which key cellular functions and molecular processes take place. Integrated with super-resolution microscopy techniques, these fabrication and structuring techniques are capable of providing previously unattainable insight into the functions of cells and specific biomolecular interactions.

Nanobiointerfaces not only hold promise for future breakthrough discoveries that further our understanding of cellular processes, but also for application in drug discovery, diagnostics and therapeutics. The creation and application of these interfaces is, by nature, highly interdisciplinary. It involves close collaboration between scientists and engineers from fields ranging from physics and electrical engineering to materials science and chemical engineering to biochemistry, biotechnology and cell biology.

With this virtual seminar we aim to create a "melting pot" to enhance and strengthen the cross-disciplinary connections that constitute the lifeline of the field, as well as to expand the language for reaching across discipline boundaries. Currently, as scientific exchange has often been reduced to established collaborations, our seminar aims to provide a forum for networking where exposure to new techniques and challenges inspires new ideas, and where the foundation for future collaborations is laid.

Introduction

Scientific Organizers:

Prof. Dr. Sebastian Springer	Jacobs University Bremen, Germany E-mail: s.springer@jacobs-university.de
Asst. Prof. Dr. Eva Sevcsik	TU Wien, Austria E-mail: eva.sevcsik@tuwien.ac.at

Administrative Organization:

Dr. Stefan Jorda Mojca Peklaj	Wilhelm und Else Heraeus-Stiftung Postfach 15 53 63405 Hanau, Germany Phone +49 6181 92325-18 Fax +49 6181 92325-15 E-mail peklaj@we-heraeus-stiftung.de Internet: www.we-heraeus-stiftung.de

Program

Program

Wednesday, 17 March 2021

Time	Speaker	Title
13:45 - 14:00	Sebastian Springer Eva Sevcsik	**Welcome & opening remarks**
	PLENARY SESSION 1 (Chair: Eva Sevcsik)	
14:00 - 14:35	Paula Mendes	**Tuning and Switching the Binding Properties of Bio-Interface Materials**
14:35 - 15:10	Khalid Salaita	**Repurposing Nucleic Acids as High-Resolution Force Sensors to Study Mechanotransduction in Living Cells**
15:10 - 15:25	Ada Cavalcanti-Adam	**Nanopatterned Materials for Receptor Crosstalk in Cell Adhesion**
15:25 - 15:40	Barbara Baird	**Micropatterned Surfaces to Investigate Assembly of Cellular Signaling Machinery**
15:40 - 16:10	*BREAK, COFFEE TABLES, SPEAKERS' TABLES*	

Time	Single Molecule Architectures via DNA Origami Nanotechnology	3D Nanostructures
Parallel Sessions	**Single Molecule Architectures via DNA Origami Nanotechnology** (Chair: Graham Leggett)	**3D Nanostructures** (Chair: Jacob Piehler)
16:10 - 16:45	Matteo Palma **DNA Origami Nanoarrays for Multi-valent Cellular Investigations with Single-Molecule Control**	Bianxiao Cui **The Role of Membrane Curvature at the Nano-Bio Interface**
16:45 - 17:00	Christof Niemeyer **Multiscale DNA Systems to Investigate Living Cells**	Jose Moran-Mirabal **Shrink and Wrinkle: Structuring of Thin Films on Thermo-Responsive Substrates for the Study of Cell-Surface Interactions**
17:00 - 17:15	Joschka Hellmeier **DNA Origami Demonstrate the Unique Stimulatory Power of Single pMHCs as T-Cell Antigens**	Karen L. Martinez **Stable Cytosolic Access of High-Aspect-Ratio Nanostructures for Novel Biological Applications**
17:15 - 20:00 (open end)	***POSTER SESSION 1,*** *COFFEE TABLES, SPEAKERS' TABLES*	

Program

Thursday, 18 March 2021

PLENARY SESSION 2 (Chair: Sebastian Springer)

14:00 - 14:35	Christelle Prinz	**Nanostructures for Probing and Transfecting Living Cells**
14:35 - 15:10	Michael Sheetz	**Mechanical Stresses Kill Tumor Cells**
15:10 - 15:25	Haogang Cai	**Nanoengineered Biomimetic Interfaces for Mechanobiology Study**
15:25 - 15:40	Enrico Domenico Lemma	**Selective Cell Adhesion on 3D Scaffolds Via Photo-Induced DNA Functionalization**
15:40 - 16:10	*BREAK, COFFEE TABLES, SPEAKERS' TABLES*	

Parallel Sessions	**Spatial, Mechanical and Chemical Cue** (Chair: Gerhard Schütz)	**Tunable Biomaterials** (Chair: Elena Martinez Fraiz)
16:10 - 16:45	Mark Schvartzman **Nanoscale Spatio-Mechanical Regulation of the Immune Signaling in Cytotoxic Lymphocytes**	Friedrich Simmel **Programming Cells and Biomaterials with Nucleic Acid Strand Displacement Processes**
16:45 - 17:00	Kheya Sengupta **AI Based Detection of Hidden Features in T Cell Architecture**	Ana Díaz Álvarez **Developing a Novel and Tunable Biomaterials Platform to Mimic the Intercellular Interface**
17:00 - 17:15	Joachim Rädler **Adhesion-Velocity Relation of Motile Cells in Confined Geometry**	Changjiang You **Nanoscopic Anatomy of Dynamic Multi-Protein Complexes at Membranes Resolved by Graphene Induced Energy Transfer**

17:15 - 17:50	Jay T. Groves	**Visualizing the LAT Protein Condensation Phase Transition in T Cell Signaling**
17:50 - 17:55	Stefan Jorda	**Wilhelm and Else Heraeus Foundation**
17:55 – 20:00 (open end)	***POSTER SESSION 2*** *COFFEE TABLES, SPEAKERS' TABLES*	

Posters

Poster Session 1 – Wed, 17 March – 17:15-20:00 h (open end)

1	Gulden Akcay	**Mimicry of ECM Stiffness Gradients in Brain-on-Chip**
2	Christine Arndt	**Microengineered Hollow Graphene Tube Systems Generate Conductive Hydrogels with Extremely Low Filler Concentration**
3	Mouhanad Babi	**Tuning the Nanotopography and Functionality of 3D-Printed Cellular Scaffolds Through Cellulose Nanocrystal Coatings**
4	Rachel Bender	**An Indestructible Tension Probe for Measuring High-Force Mechanical Events in Cells**
5	Eider Berganza Eguiarte	**Fabrication of Multiplexed Curved Phospholipid Membrane Platform**
6	Veronika Brumovska	**Determination of the Actual Size of Proteins in the Live Cell Plasma Membrane**
7	Ignasi Casanellas	**Dynamics of Stem Cell Migration and Condensation on Nanopatterned Adhesive Ligands**
8	Joel Christian	**Imaging Membrane Deformation During Nanoparticle Uptake at the Ventral Side**
9	Brendan Deal	**Engineering DNA-Functionalized Nanostructures to Bind Nucleic Acid Targets Heteromultivalently with Enhanced Avidity**
10	Yagmur Demircan Yalcin	**Biophysical Cues Affect Neural Network Dynamics**
11	Yuxin Duan	**Democratizing Mechanobiology with Mechanically Triggered Hybridization Chain Reactions**

12	Roland Hager	**Biomolecule Micropatterning on Different Polymeric Substrates and Its Applicability for Quantitative Fluorescence Microscopy**
13	Ingrid Hartl	**Analysis of FGFR3 Signaling Strength Using Micropatterning**
14	Yuesong Hu	**Microparticle Molecular Tension Sensors for Mapping Cell Mechanics in Non-Planar Geometries and for High-Throughput Quantification**
15	Martin Humenik	**Nanostructured Surfaces Via Self-Assembly of DNA-Spider Silk Conjugates**
16	Nouria Jantz	**Micropatterned Arrays of Peptide-Receptive MHC Class I Proteins**
17	Peter Lanzerstorfer	**A Visual Immunoprecipitation Assay for Live-Cell Profiling of Cytosolic Protein Complexes on Micropatterned Substrates**
18	Graham Leggett	**Its Quantum Biology, Jim, But Not as We Know It**
19	Victoria Levario Diaz	**1D Nanopatterned Integrin Ligand Surface to Evaluate Stick-Slip Dynamics**
20	Ostap Lishchynskyi	**Whether Is Impact of the Low Concentration of the CaCO3 Nanoparticles Embedded in Non-Fouling Temperature-Responsive POEGMA Coatings on Different Cell Lines?**
21	Cristina Lo Giudice	**Force-Distance Curve-Based AFM and Single Cell Approaches to Probe Ligand-Receptor Interactions and Mechanics**
22	Chih-Hao Lu	**Membrane Curvature Regulates the Spatial Distribution of the Glycocalyx Proteins**

23	Rong Ma	**Ensemble Measurement of TCR-pMHC Force Lifetime by DNA Hybridization Kinetics**

Poster Session 2 – Thu, 18 March – 17:55-20:00 h (open end)

24	Monalisa Mishra	**Study of Interaction of Nanomaterials with Cells and Tissues Using Drosophila Melanogaster**
25	Zeinab Mohamed	**Clinically Relevant Bacteria Outer Membrane Models for Antibiotic Screening Applications**
26	Lital Mordechay	**Mechanical Regulation of the Cytotoxic Activity of Natural Killer Cells**
27	Ulrike Müller	**Implementation of DNA Surface Technology on Large-Area Micropatterned Substrates for Interaction Analysis in Live Cells**
28	Melissa Nakamoto	**Expansion Microscopy at the Nano-Bio Interface**
29	Svyatoslav Nastyshyn	**Non-Cytotoxic, Temperature-Responsive and Antibacterial POEGMA Based Nanocomposite Coatings with Silver Nanoparticles**
30	Andreas Neusch	**Magnetic Manipulation Strategies Towards Cell Signaling Studies**
31	Federica Pennarola	**Molecular Forces Involved in Clathrin-Mediated Endocytosis of Nanoparticles and Viruses**
32	Michael Philippe	**Nanoscopic Organization of Wnt Signalsomes for Interrogation and Manipulation of Downstream Signaling**
33	Allison Ramey-Ward	**Mechanical Stimulation with Nanoscale Actuators Provides Scalable Spatiotemporal Control of Muscle Cell Biology**
34	Andreas Rohatschek	**Investigation of Nanoscale Collagen Films by SFA**

Poster Session 2 – Thu, 18 March – 17:55-20:00 h (open end)

35	Harekrushna Sahoo	**Conformation and Dynamics of Bone Morphogenetic Protein (BMP-2): Role of Extracellular Matrix (ECM) Based Nanoparticles and Minerals**
36	Lukas Schrangl	**A FRET-Based Sensor for Probing Forces Exerted by Single T Cell Receptors on their Ligands**
37	Kevin Scrudders	**Reconstituting PLCβ Domain Function Using Supported Lipid Bilayers**
38	Valentina Serrano	**Design of Experiments to Fabricate Hydrogels by Visible Light Photopolymerization Using a 3D Bioprinter**
39	Ananya Shrivastava	**Investigating the Influence of Biophysical Properties of the Environment on Tumor Cell Dormancy**
40	Tiffany Tang	**Development of an Electroactive Platform for Detection of Virus Fusion to Host Membranes**
41	Ana Teixeira	**DNA Nanotechnology to Map and Control the Nano-Organisation of Membrane Proteins**
42	Esti Toledo	**Nanochip for Personalized Assessment of Checkpoint Immunotherapy**
43	Nikki Wanders	**Substrate Stiffness Effects on Neuronal Cell Culture in Vitro**
44	Gregor Weisgrab	**Upscaling Modular Tissue Engineering**
45	Chunting Zhong	**Microchannel Cantilever Spotted Sensor Arrays for Highly Affinitive Indicator-Displacement Assays**

Abstracts of Lectures

(in alphabetical order)

Micropatterned Surfaces to Investigate Assembly of Cellular Signaling Machinery

Jordan Mohr, Meraj Ramezani, David Holowka and Barbara Baird
Department of Chemistry & Chemical Biology, Cornell University, Ithaca, NY USA

Cells are poised to respond to their physical environment and to chemical stimuli in terms of collective molecular interactions that are regulated in time and space by the plasma membrane and its connections with the cytoskeleton and intracellular structures. Small molecules may engage specific receptors to initiate a transmembrane signal, and the surrounding system efficiently rearranges to amplify this nanoscale interaction to microscale assemblies, yielding a cellular response that often reaches to longer length scales within the organism. A striking example of signal integration over multiple length scales is the allergic immune response. IgE receptors (FcεRI) on mast cells are the gatekeepers of this response, and this system has proven to be a valuable model for investigating receptor-mediated cellular activation. My talk will describe our implementation of surfaces that are micropatterned with antigen/agonist to define the localization of receptors and thereby delineate co-localization or exclusion of fluorescently-labeled signaling components after receptors are engaged.[1] We use a polymer lift-off method[2] to deposit agonist, either immobilized on the surface or mobile within a lipid bilayer.[3] The micrometer-scale clustering of IgE-FcεRI (or, in a separate study, receptors for epidermal growth factor[4]) serves to stabilize the assembly of signaling components that otherwise may engage transiently. The defined, ordered array of these regions allows straightforward quantification of signaling, integrin and cytoskeletal components,[3] as well as exocytotic events that may be triggered to occur locally.[5] In recent studies we found that stabilization of IgE-FcεRI on the micrometer scale does not preclude examination of sub-micron localization or exclusion of membrane components, enabling a higher-resolution evaluation of these physiologic events.

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Nanoengineered biomimetic interfaces for mechanobiology study

Haogang Cai[1]

[1] *Tech4Health Institute and Department of Radiology, NYU Langone Health, New York, NY, USA*

In the last few decades, it has become increasingly evident that besides the DNA-encoded information, extracellular mechanical factors play an important role in determining the biological processes, but there is still a major gap in our understanding of the molecular mechanisms in the critical size ranging between molecular and cellular scale. In this talk, I will present nanoengineered biomimetic interfaces for mechanobiology study at the single-molecule level. By borrowing the state-of-the-art nanofabrication technology from the semiconductor industry, we create molecular architectures on microscope slides as biochips, an analogue of transistor arrays on silicon IC chips. These biochips can serve as a single-molecule breadboard to mimic the extracellular matrix or cell surfaces, and probe the cellular mechanotransduction signaling with the ultimate resolution.

We focus on the effects of geometric organization of transmembrane receptors on critical cell behavior and functions, such as adhesion formation [1] and immune response [2]. The findings highlighted a more significant role for ligand geometry over ligand density. In a fibrous extracellular matrix mesh, stable integrin nanoclusters bridge single-molecule fibers by recruiting activated but unliganded integrins, where two-dimensional arrangement of thin fibers is necessary. We also discovered three-dimensional geometric underpinnings of T cell activation, which support roles for both receptor clustering and kinetic-segregation model. These findings will provide both theoretical guidance and material platforms for cell engineering in novel therapeutics, such as adoptive cell therapy.

References

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Nanopatterned materials for receptor crosstalk in cell adhesion

Q. Wei[1], F. Posa[1], E. A. Cavalcanti-Adam[1]

1Max Planck Institute for Medical Research, Heidelberg, Germany

The crosstalk between different receptor types at the interface with the extracellular environment is crucial for cell adhesion, migration and differentiation. Nanopatterned surfaces which combine adhesive ligands and growth factors allow to study how local changes in the extracellular environment regulate cell responses through specific receptor-ligand interactions.

For adhesion to the extracellular matrix, integrin lateral clustering strongly influences cell adhesion dynamics and forces [1]. The nanoscale presentation of integrin ligands combined with growth factors, namely BMP-2 and BMP-6, modulates not only the specific interaction with different integrin types [3], but also the osteogenic differentiation of cells [4, 5]. Such nanopatterning approaches can be also applied to hydrogels of varying stiffness to elucidate the interdependency of mechanotransduction and differentiation signaling [6].

References

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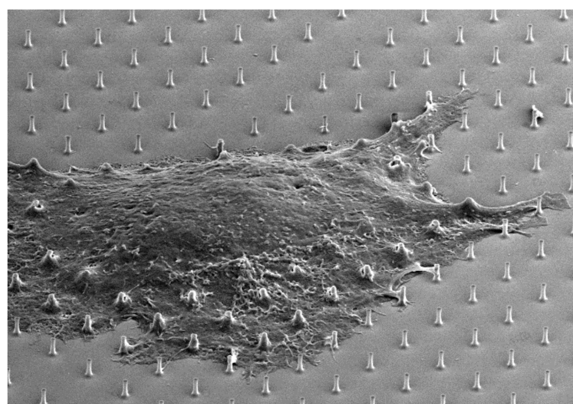
Title: The role of membrane curvature at the nano-bio interface

Bianxiao Cui, Department of Chemistry, Stanford University, USA

Abstract: Membrane curvature in the range of tens to hundreds of nanometers is involved in many essential cellular processes. At the cell-matrix interface, where the cells make physical contact with extracellular matrices, the membrane may be locally deformed by matrix topography or mechanical forces, and this deformation may actively regulate signal transmission through the interface. We explore nanofabrication to engineer vertical nanostructures protruding from a flat surface. These nanostructures deform the plasma membrane to precisely manipulate the location, degree, and sign (positive or negative) of the interface curvature in live cells. We found that the high membrane curvature induced by vertical nanostructures significantly affects the distribution of curvature-sensitive proteins and stimulates intracellular processes in live cells [1-4]. Our studies show a strong interplay between biological cells and nano-featured surfaces and reveal curvature-dependent molecular mechanisms in cells.



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Developing a novel and tunable biomaterials platform to mimic the intercellular interface

A. Díaz Álvarez[1], M.K.L. Han[1], J. Zhang[1], M. Rübsam[2], C. Niessen[2], A. del Campo[1]

[1] *Dynamic Biomaterials, Leibniz Institute for New Materials, Saarland University, Saarland, Germany*

[2] *Department of Dermatology, University of Cologne, Cologne Excellence Cluster for Stress Responses in Ageing-associated diseases (CECAD), Cologne, Germany*

Cells form specialized types of adhesions with their surrounding environment, amongst them the integrin-based focal adhesions (cell-ECM) and cadherin-based adhesions (cell-cell) [1]. It has been shown that both of these cell-adhesion types are able to sense mechanical environmental cues and influence cell behavior [2, 3]. Moreover, there is evidence that they can stablish a close mechanical crosstalk, the nature of which seems to be rather complex, with some studies showing a competitive relationship [4, 5] and others a cooperation of sorts [6]. To further study the nature of the dialogue between integrin and cadherin-based adhesions, a platform that allows for the fine tuning of the mechanical and chemical properties of two orthogonal interfaces is needed. Using poly(acrylamide), an inexpensive, cytocompatible material widely used for mechanobiology studies [7], our group has developed a 2.5D artificial microenvironment that allows for independent, orthogonal tuning of both mechanical and chemical properties. We achieve this through a soft molding method involving the separate polymerization of two hydrogel layers. Through orthogonal coupling chemistries, the ECM and cell-cell interfaces can then be mimicked. This platform has the potential to become a powerful tool for studying mechanosensing at the cell adhesion level, as well as for unraveling the intricacies of the crosstalk stablished between cell-cell and cell-matrix adhesions.

References

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Visualizing the LAT protein condensation phase transition in T cell signaling

Jay T. Groves

Department of Chemistry, University of California Berkeley

Linker for Activation of T cells (LAT) is an intrinsically disordered, membrane-anchored protein that serves as a scaffold to assemble key downstream signaling molecules in the T cell receptor pathway. In reconstituted experiments, it has been recently demonstrated that the activation process of LAT involves a protein condensation phase transition[1,2], and this may further control downstream signaling activity[3]. In this presentation, I will describe a series of single molecule imaging experiments performed in live T cells[4] aimed at visualizing the LAT phase transition and resolving how distinctive features of the phase transition (as opposed to a more linear assembly process) may contribute functional advantages to the T cell signaling system.

References

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DNA origami demonstrate the unique stimulatory power of single pMHCs as T-cell antigens

Joschka Hellmeier[1], Rene Platzer[2], Alexandra S. Eklund[3,4], Thomas Schlichthärle[3,4], Andreas Karner[5], Viktoria Motsch[1], Magdalena C. Schneider[1], Elke Kurz[6], Victor Bamieh[1], Mario Brameshuber[1], Johannes Preiner[5], Ralf Jungmann[3,4], Hannes Stockinger[2], Gerhard J. Schütz[1], Johannes B. Huppa[2], Eva Sevcsik[1]

[1] Institute of Applied Physics, TU Wien, Vienna, Austria
[2] Center for Pathophysiology, Infectiology and Immunology, Institute for Hygiene and Applied Immunology, Medical University of Vienna, Vienna, Austria
[3] Max Planck Institute of Biochemistry, Martinsried, Germany
[4] Faculty of Physics and Center for Nanoscience, Ludwig Maximilian University, Munich, Germany
[5] University of Applied Sciences Upper Austria, Linz, Austria
[6] Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK

T cells recognize the presence of even a single foreign peptide-loaded major histocompatibility complex (pMHC) on the surface of antigen presenting cells (APC) even though their T-cell antigen receptors (TCR) bind with micromolar affinity. How they achieve this is still poorly understood, yet indirect evidence pointed to spatial antigen arrangement on the APC surface as a critical factor for this recognition process.
To examine this, we engineered an APC-mimicking biointerface with dual functionality: first, it should generate defined exclusion zones around individual ligands to isolate them as they cluster during T-cell activation; second, it should permit the directed pre-organization of single ligand molecules with nanometer precision. To this end, we employed rectangular DNA origami platforms anchored to fluid-phase planar supported lipid bilayers (SLB) and functionalized with either one of two different types of TCR ligands: high-affinity TCR-reactive monovalent single chain antibody fragments (scF_V) to serve as templates for the organization of TCRs, or low-affinity antigenic pMHCs as the natural ligand.
When confronting CD4+ effector T cells with this novel biointerface we found that T-cell activation via anti-TCR scF_V requires close proximity ($\leq 20nm$) of ligands within units of at least two molecules, indicating that TCRs must be antibody-engaged in close proximity to initiate a potent immune response. This held no longer true for the physiological TCR ligand, cognate pMHCs, which were well capable of stimulating T cells as well-isolated entities.
Together, our data indicate that early T-cell signaling emerges from small assemblies of triggered TCRs, which can be formed either by prolonged TCR:ligand engagement of closely spaced TCRs or by repeated short-lived interactions via single cognate pMHC molecules.

Selective cell adhesion on 3D scaffolds via photo-induced DNA functionalization

E.D.Lemma[1], R.Tabone[2], K. Richler[1], A.K.Schneider[3], C.Niemeyer[3], C.Bizzarri[2], and M.Bastmeyer[1]

[1]Zoological Institute, Cell and Neurobiology, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany
[2]Dept. of Organic Chemistry, KIT, Karlsruhe, Germany
[3]Inst. for Biological Interfaces, KIT, Karlsruhe, Germany

Functionalization of microstructures with biologically relevant molecules has allowed for studying cells under controlled conditions, even at single-cell resolution. However, low specificity and low spatial selectivity usually affect current functionalization techniques. Moreover, current 2D microfabrication techniques prevent from studying cells in a more physiologically-resembling 3D environment [1].
Here we fabricate 3D microscaffolds with a direct laser writing technology (i.e., two-photon lithography) and locally decorate them with single-strand DNA (ssDNA) via light-induced click chemistry.
In particular, we first polymerize acrylate-based photosensitive materials via 2PL and subsequently irradiate the patterns with a 405nm laser in the presence of maleimide-biotin, which reacts with the acrylate. Then, we incubate with streptavidin and a biotinylated oligonucleotide (Fig.1). The so-created multi-molecular complex (i) is shown to be stable under several conditions, (ii) can be obtained with two different photosensitive materials for 2PL, and (iii) can be used to locally functionalize on 3D structures (Fig.2a and 2b). Finally, we include cholesteryl-TEG complementary ssDNA into MCF7 cell membranes, and allow for base pair hybridization in culture-like conditions [2]. Our results demonstrate that these DNA-functionalized cells selectively adhere to the structures only where the complementary DNA-functionalization had been carried out (circles in Fig.3, surrounded by non-functionalized squares).
Therefore, we provide a highly specific and spatially resolved functionalization technique allowing for precise cell positioning in 3D.

Fig.1

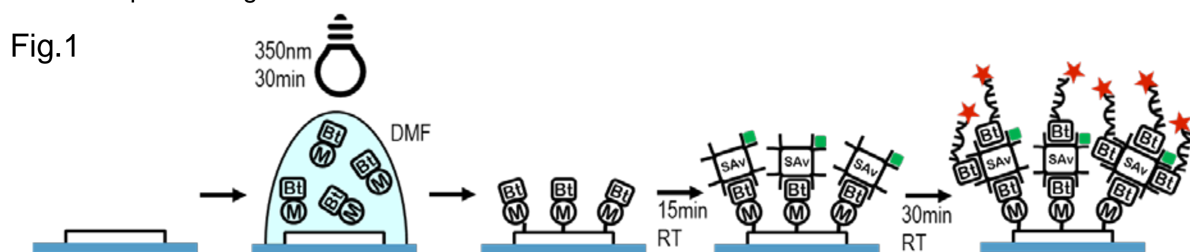


Fig.2

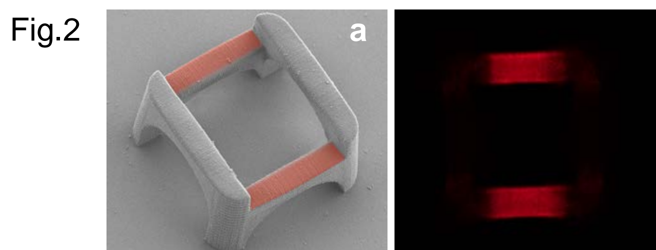
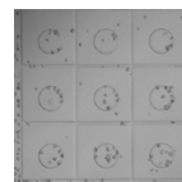


Fig.3



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Stable cytosolic access of high-aspect-ratio nanostructures for novel biological applications

K.L. Martinez[1]

[1]*Dpt of Chemistry, University of Copenhagen, Denmark*

High-aspect-ratio nanostructures have been shown as powerful tool for single cell manipulation and investigation. They have been mostly used for extra-cellular applications (e.g. cell guidance, modification of the topography of the plasma membrane) while their potential for intra-cellular applications is only emerging and based on transient delivery of molecules. Stable and direct access to the cytosol remains a bottleneck limiting the development of novel biological applications. During my talk, I will address this challenge and show that it can be overcome.

Tuning and switching the binding properties of bio-interface materials

Paula M Mendes

School of Chemical Engineering, University of Birmingham,
Edgbaston, Birmingham, B15 2TT, UK.
Email: p.m.mendes@bham.ac.uk

Inspired by nature's responsive mechanisms and our increased capability to manipulate matter at the molecular level, the time has come for us to embrace the design and construction of bio-interface materials with increasing dynamic, functional complexity. The lecture will highlight how the availability of sophisticated new experimental techniques and tools of nanotechnology can be used to create stimuli-responsive interfaces for fundamental cellular studies, regenerative medicine and on-demand sensing. In addition, progress on molecularly engineered surfaces for protein immobilization and glycan recognition and their application in earlier and more accurate diagnoses of diseases, such as cancer, will be described. The lecture will also discuss how we need to continue enhancing the functional complexity at the bio-interface to fully address the current challenges in biotechnology and biomedicine.

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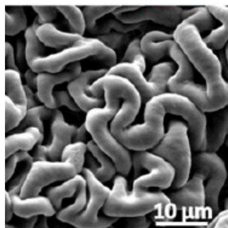
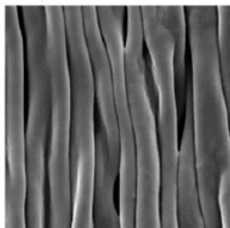
Shrink and Wrinkle: Structuring of thin films on thermo-responsive substrates for the study of cell-surface interactions

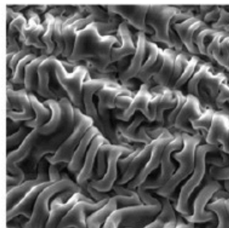
S. Makaremi,[1] K.J. De France,[2] M. Babi,[3] D.M.E. Bowdish,[4] T. Hoare,[2] E.D. Cranston,[2,5] and J.M. Moran-Mirabal[1,3]

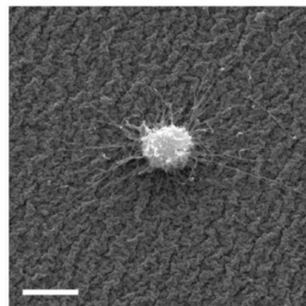
[1]School of Biomedical Engineering, McMaster University, Hamilton, ON, Canada
[2]Department of Chemical Engineering, McMaster University, Hamilton, ON, Canada
[3]Department of Chemistry and Chemical Biology, McMaster University, Hamilton, ON, Canada
[4]Department Molecular Medicine and Pathology, McMaster University, Hamilton, ON, Canada
[5]Department of Chemical and Biological Engineering, University of British Columbia, Vancouver, BC, Canada

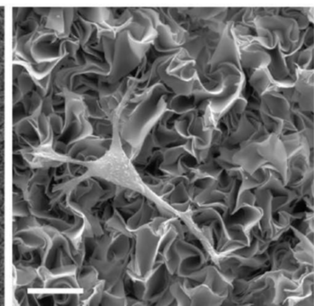
In nature, cellular microenvironments present a variety of physical and biochemical stimuli at the micro- to nanoscale that have important effects on cell adhesion, migration, proliferation, morphology and overall cellular function. However, the details of how surface topography at these scales impacts morphology and function remain unclear. In part, this is due to the lack of simple methods to controllably structure surfaces with features spanning the micron to nanoscale. In this presentation, we introduce a simple bench-top approach, based on the thermal shrinking of thermo-responsive substrates, for the controllable wrinkling of glassy and hydrogel thin films. We will show using this method it is possible to produce surfaces with random or oriented topographical features in the micron to nanometer scale. Furthermore, by employing adhesive masks during the thin film deposition process, we also show that structured surfaces with feature size gradients can be fabricated. The impact of these surfaces on cell morphology, membrane receptor mobility and cell function will be discussed. It is anticipated that the precise control over both feature size and topography that this simple fabrication method provides will add a level of functionality that is required to understand fundamental cell-surface interactions.











Multiscale DNA systems to investigate living cells

Christof M. Niemeyer

Karlsruhe Institute of Technology (KIT), Institute for Biological Interfaces (IBG-1), Herrmann-von-Helmholtz Platz 1, D-76344 Eggenstein-Leopoldshafen, Germany.

The advent of DNA microarray technology in the course of the human genome project in the late 1980s has led to the evolution of sophisticated DNA-functionalized solid substrates, which are nowadays routine tools for fundamental and applied research in biology and medicine. However, the versatility of DNA biochips goes far beyond the established applications in genotyping and expression profiling, because their capability for highly parallel, site-directed immobilization of complementary nucleic acids can be harnessed to assemble complex surface architectures comprised of colloidal materials and proteins. While this approach enables novel sensor platforms for protein and small-molecule analysis, the full potential of DNA surfaces can be exploited by implementation of structural DNA nanotechnology.

We have previously applied this DNA chip-based approach to investigate cell adhesion and the recruitment of transmembrane proteins in living cells. By combining DNA micro- and nanostructures, we have now developed “multiscale origami structures as interface for cells” (MOSAIC). This tool allows one to present ligands to living cells on surfaces with a full control over their stoichiometry and nanoscale orientation, thereby enabling to address fundamental questions in cell signalling which cannot be tackled by conventional technologies.

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DNA Origami Nanoarrays for Multi-valent Cellular Investigations with Single-Molecule Control

Matteo Palma
[1] Department of Chemistry, Queen Mary University of London, London, U.K

The controlled organization of individual molecules and nanostructures with nanoscale accuracy is of great importance in the investigation of single-molecule events in biological and chemical assays, as well as for the fabrication of the next generation optoelectronic devices. DNA has been shown to be an ideal structural material for this.

Here we will show discuss a facile strategy combining the programming ability of DNA as a scaffolding material with a one-step lithographic process.[1] can be employed to fabricate biomimetic nanoarrays that permit the multivalent investigation of ligand−receptor molecule interactions in cancer cell spreading,[2] as well as the study of cardiomyocyte adhesion and maturation,[3] with nanoscale spatial resolution and single-molecule control.

In particular, we designed multi-valent DNA nanoplatforms and chemisorbed them on a nanopatterned surface so to co-localize an $\alpha v\beta 6$-integring specific peptide and the epidermal growth factor (EGF) within 60 nm (optimal integrin spacing) in array configurations on the same biochip. We will demonstrate how the platform developed allowed us to study, with single-molecule control, integrin-dependent responses and their cooperation with EGF in the adhesion of epidermal cancer cell. [2]

Moreover, we demonstrate how the platform can be used to interrogate the role of integrin clustering in cardiomyocyte adhesion and maturation;[3] we find previously unknown clustering behaviour of different integrins, further outlining the importance for such customisable platforms for future investigations of specific receptor organisation at the nanoscale.

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NANOSTRUCTURES FOR PROBING AND TRANSFECTING LIVING CELLS

Christelle N. Prinz

Division of Solid State Physics and NanoLund, Lund University, 221 00 Lund, Sweden

Interacting with cells in a minimally invasive manner is key to performing longitudinal studies in biomedicine. One example where minimally invasive interaction with cells is important is cellular mechanosensing. Mechanical forces are exerted in many crucial cellular processes and the investigation of cellular forces is expected to yield important information on, e.g., cancer formation and epithelial to mesenchymal transition (metastasis). In order to extract useful information from force measurements, one must be able to measure traction forces on multiple cells, over long periods of time without any detrimental effects on the cells. This can be achieved by using nanowires in a bed-of-nails configuration, measuring traction forces exerted by cells cultured on top of the nanowires [1,2].

Another type of intervention consist of injecting material inside cells, either to probe cells, or to change the cell phenotype (transfection). Current delivery methods such as viral vectors electroporation and liposome delivery (lipofection) have many drawbacks. Viruses suffer from being costly, can be unsafe due to insertional mutagenesis, and are limited in cargo size. Electroporation and lipofection, suffer from low cell viability and efficiency. To address this issues, nanostraws can be used to deliver cargos inside cells[3–5].

Here, a review of our work using nanowires and nanostraws for developing mechanosensors and cell transfection tools will be presented.

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Adhesion-Velocity Relation of Motile Cells in Confined Geometry

C. Schreiber[1], B. Amiri[2], J.C.J. Heyn[1], J.O. Rädler[1] , and M. Falcke[2]

[1]Institute, Faculty of Physics and Center for NanoScience, Ludwig-Maximilians-Universität München, 80539 Munich, Germany
[2] MaxDelbrück Center for Molecular Medicine in the Helmholtz Association, 13125 Berlin, Germany; Department of Physics, Humboldt University, 12489 Berlin, Germany

In eukaryotic migration, protrusion dynamics, cell velocity and shape exhibit spatio-temporal pattern that are controlled by the underlying cytoskeleton organization. In this context, artificial micro-pattern are convenient platforms to study cell motion as they provide defined geometric boundaries and surface chemistry. We study motion of MDA-MB-231 cells on microlanes with fields of alternating Fibronectin densities to address the biphasic adhesion-velocity relation. We derive a mathematical model from the leading-edge force balance and the force-dependent polymerization rate that reproduces the steady state velocity as a function of Fibronectin density. At transitions between different Fibronectin densities, steady motion is perturbed and leads to changes of cell length and front and rear velocity. Cells exhibit an intrinsic length set by adhesion strength, which, together with the length dynamics, suggests a spring-like front–rear interaction force.

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Repurposing nucleic acids as high-resolution force sensors to study mechanotransduction in living cells

Khalid Salaita[1]

Emory University, Department of Chemistry
Atlanta, Georgia, USA, 30322
k.salaita@emory.edu

Cells are highly dynamic structures that are constantly converting chemical energy into mechanical work to pull and push on one another and on their surroundings. These pulls and pushes are mediated by molecular forces at the scale of tens of piconewtons. For context, 7 pN applied a distance of 1 nm is ~1 kcal/mol. Nonetheless, these forces can have profound biochemical consequences. For example, the rapidly fluctuating forces between immune cells and their targets can drastically tune immune response and function. Despite the importance of mechanics there are limited methods to study forces at the molecular scale and particularly within the context of living cells. In this talk, I will discuss my group's efforts at addressing this gap in knowledge by developing tools to map and manipulate the molecular forces applied by cells. I will describe the development of a suite of molecular tension probes. Tension probes are modular and can be engineered using PEG polymers, oligonucleotides, and proteins. The latest generation of tension probes employ nucleic acids, which provide significant improvements in resolution and allow one to employ signal amplification strategies. I will show exciting new advances that harness fluorescence polarization spectroscopy (1) and super-resolution imaging (2) to provide the highest resolution maps of cell traction forces reported to date. I will also describe the application of these probes in the study of platelet activation, podosome formation (3), and T cell receptor mechanobiology. Finally, armed with these new tools, I will demonstrate that molecular forces not only give rise to tissue architecture but also to boost the fidelity of information transfer between cells. We dubbed this mechanism *mechanical proofreading* in analogy to the kinetic proofreading model used explain the extraordinary fidelity of DNA replication and protein expression. I will show examples of mechanical proofreading in adaptive T cell immunity and platelet coagulation.

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2. Nature Methods, volume 17, pages 1018–1024 (2020)
3. Nature Communications volume 10, Article number: 4507 (2019)

Nanoscale spatio-mechanical regulation of the immune signaling in cytotoxic lymphocytes.

Mark Schvartzman

[1]*Department of Materials Engineering, Ben-Gurion University, Beer-Sheva, Israel*

It has been long known that cytotoxic lymphocytes – the sentinels of our immune system – differentiate between pathogens and healthy cells by sensing environmental chemical cues, which are delivered by the ligands expressed on the surface target cells. Yet, it is becoming progressively clear that lymphocytes sense also physical environmental cues, such as ligand arrangement, mechanical stiffness, and topography. In the first part of my talk, I will review our recent study of the role of the ligand arrangement in the immune function of Natural Killer (NK) cells, using nanoengineered stimulating platforms based on patterned arrays of ligands. The first generation of such platforms was based on arrays of nanoimprinted metallic nanodots functionalized with activating ligands, which allowed us to discover the minimal spatial requirement of ~ 1 ligand per sq. micron needed for the activation of NK cells[1]. The next, more advanced generation of arrays came to examine how the segregation between activating and inhibitory ligands affects the inhibition of activating signaling in NK cells[2]. The platform was based on ordered arrays of nanodots of two metals selectively functionalized with activating and inhibitory ligands, whose segregation was systematically tuned between 0 nm to 40 nm. Surprisingly, we found that inhibition efficiency increased with the spacing between the ligands within the probed range, and rationalized this finding by physical modeling of the ligand-receptor binding kinetics.

In the second part of my talk, I will review our recent study of the role of environmental elasticity and topography in the function of cytotoxic lymphocytes. Stimulation of NK cells on planar elastomers functionalized with activating ligands revealed a bell-shape trend of activation vs. elastic modulus[3]. A more complex stimulating platform was based on ligand functionalized nanowires to designed to deliver both chemical, nano-topographical, and mechanical cues, whose combination produced an enhanced immune response of NK cells[4]. To separately reveal the effect of each cue, we recently stimulated NK cells and CD8+ T cells on nanowires with varied length and bending moduli and found that these physical parameters of nanowires greatly affects the signaling and the immune function of the lymphocytes. Overall, our study work provides an important insight into the way the physical cues regulate the function of NK cells and T cells.

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AI based detection of hidden features in T cell architecture

A. Nassereddine[1,2], A. Abdelrahman [1,2], E. Benard[1],

F. Bedu [1], I. Ozerov [1], L. Limozin [2] K. Sengupta [1]

[1]Aix Marseille University, CNRS, Centre Interdisciplinaire de Nanoscience de Marseille (CINaM), 13009 Marseille, France.
[2] Aix Marseille University, CNRS, INSERM, Laboratory Adhesion Inflammation (LAI), 13009 Marseille, France.

Protein-patterning has emerged as a powerful means to interrogate adhering cells. Yet, there are no standard tools to apply a sub-micron periodic stimulus or to analyse the response. We propose a technique combining electron-beam lithography and surface functionalization to fabricate nano-patterns compatible with advanced imaging. The repetitive pattern facilitates a deep-learning algorithm to reveal that T cells organize their membrane and actin network differently depending on whether the ligands are clustered or homogeneously distributed - an effect invisible to the unassisted human eye even after extensive image analysis. Our integrated fabrication and analysis tool-box should be useful for exploring general correlation between a spatially-structured sub-cellular stimulation and the cellular response.

References

Mechanical Stresses Kill Tumor Cells

Michael Sheetz, Ph.D.

Welch Professor of Biochemistry
Molecular MechanoMedicine Program
Biochemistry and Molecular Biology
University of Texas Medical Branch
Galveston, TX, USA

Tumor formation correlates with repeated damage or inflammation and involves a release of growth control for adult tissues, particularly evident as tumor cell growth on soft surfaces. We find that the depletion of rigidity sensors causes transformed cell growth even in normal cells and restoration of sensors causes rigidity-dependent growth even in tumor cells (Wolfenson et al., 2016; Yang et al., 2020). The rigidity sensor complex (about 2 micrometers in length) contracts matrix adhesions by ~100nm; and if the force generated is greater than ~25 pN, then normal cells can grow. However, if the surface is soft, then the cells apoptose by DAPK1 activation (Qin et al., 2018). Surprisingly, mechanical stretch of transformed tumor cells activates apoptosis through a calpain-dependent process downstream of Piezo1 and the ER-mitochondrial stress pathway (Tijore et al., 2018). Recently, we found that low level ultrasound will activate the apoptosis of tumor cells from many tissues and transformed normal cells in vitro and in the chick embryo (Tijore et al., 2020). Thus, a variety of mechanical stresses can damage transformed cells from widely different tissues in correlation with the changes in the organization of the cytoplasm upon transformation (Sheetz, 2019). These results are consistent with other studies of the effects of mechanical stresses including exercise on tumor cells and suggest that ultrasound treatment can aid in cancer therapy.

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Programming cells and biomaterials with nucleic acid strand displacement processes

Friedrich C. Simmel

Physik Synthetischer Biosysteme, Physik-Department E14, TU München

The displacement of one of the strands of a DNA or RNA duplex by an "invader" strand via toehold-mediated branch migration is one of the most often used processes in dynamic DNA nanotechnology, and has been applied to develop a wide range of DNA-based nanomachines and sensors. In the present talk, we will discuss applications of these processes in the context of DNA-programmable biomaterials, where we will show how strand displacement can be used to spatially address DNA-functionalized gel particles [1] and 3D-printed hydrogels [2]. Furthermore, we will show that strand displacement can also be used to control RNA-based gene regulation processes, e.g., in the context of synthetic riboregulators and conditional guide RNAs for CRISPR processes [3].

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MHC class I immune receptors form noncovalent, transient heavy chain dimers at the cell surface

Cindy Dirscherl[1,x], Sara Löchte[2,x], Zeynep Hein[1], Janine-Denise Kopicki[3], Antonia Regina Harders[1], Noemi Linden[1], Julian Weghuber[4], Maria Garcia-Alai[5,6], Charlotte Uetrecht[3,7], Martin Zacharias[8], Jacob Piehler[2,y], Peter Lanzerstorfer[6,y], and Sebastian Springer[1,y]*

[1]Department of Life Sciences and Chemistry, Jacobs University Bremen, Germany;
[2]Department of Biology and Center of Cellular Nanoanalytics, University of Osnabrück, 49076 Osnabrück, Germany;
[3]Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany;
[4]University of Applied Sciences Upper Austria, 4600 Wels, Austria;
[5]European Molecular Biology Laboratory, Hamburg Outstation, Hamburg, Germany;
[6]Centre for Structural Systems Biology, Hamburg, Germany;
[7]European XFEL, Schenefeld, Germany;
[8]Physics Department, Technical University of Munich, Garching, Germany.
[x]Equal contribution.
[y]Equal contribution.

Major histocompatibility complex class I proteins (MHC I) sit in the plasma membrane of mammalian cells to present antigenic peptides to T cells. When they lose the antigenic peptide and the light chain beta-2 microglobulin (beta-2m) to become so-called 'free heavy chains', then they can associate with one another, forming homotypic oligomers on the plasma membrane that are insufficiently understood. Here, we investigate this homotypic interaction of MHC I free heavy chains by combining a printed antibody micropattern assay with fluorescence recovery after photobleaching (FRAP) and with single molecule co-tracking in order to elucidate their molecular structure, abundance, and dynamics. We find that MHC I free heavy chain complexes are dimeric, transient, non-covalent, and mediated by the alpha-3 domain. Free heavy chain interaction correlates with a decrease in the diffusion coefficient and an increase in the number of immobile molecules at the cell surface. Molecular docking and dynamics simulations suggest that in the complexes, the alpha-3 domain of one FHC binds to another free heavy chain in a manner similar to the beta-2m light chain. We propose distinct functions of these MHC I free heavy chain dimers in signaling and in the endocytic sorting at the end of their lifespan.

Nanoscopic anatomy of dynamic multi-protein complexes at membranes resolved by graphene induced energy transfer

Nadia Füllbrunn[1], Zehao Li[1,2], Lara Jorde[1], Changyuan Yu[2], Carola Meyer[1], Jörg Enderlein[3], Christian Ungermann[1], Jacob Piehler[1] & Changjiang You[1]

[1] *Osnabrück University, 49076 Osnabrück, Germany.*
[2] *Beijing University of Chemical Technology, 100029 Beijing, China.*
[3] *Georg August University, 37077 Göttingen, Germany.*

Insight into the conformational organization and dynamics of proteins complexes at membranes is essential for our mechanistic understanding of numerous key biological processes in living cells. Here, we introduce graphene-induced energy transfer (GIET) to probe axial orientation of arrested macromolecules at lipid monolayers. Based on a calibrated distance-dependent efficiency within a dynamic range of 25 nm, we analyzed the conformational organization of protein complexes involved in tethering and fusion at the lysosome-like yeast vacuole. We observed that the membrane-anchored Rab7-like GTPase Ypt7 shows conformational reorganization upon interactions with effector proteins. Ensemble and time-resolved single molecule GIET experiments revealed that the HOPS tethering complex, when recruited via Ypt7 to membranes, is dynamically alternating between a "closed" and an "open" conformation, with the latter possibly interacting with incoming vesicles. The results shed new light on the structural dynamics of HOPS in living cells. Our work highlights GIET as a unique spectroscopic ruler to reveal the axial orientation and dynamics of macromolecular complexes at biological membranes with sub-nanometer resolution. The GIET approach could be combined with electron microscopy to achieve a detailed structural picture of proteins in action. By controlled surface interfacing with cells, GIET analysis to probe the axial organization of signaling complexes in the plasma membrane can be envisaged.

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Abstracts of Posters

(in alphabetical order)

Mimicry of ECM stiffness gradients in brain-on-chip

G. Akcay[1] and R. Luttge[1]

[1]*Eindhoven University of Technology, Eindhoven, The Netherlands*

Hydrogels have been explored to mimic three-dimensional (3D) tissue microenvironments in-vitro thanks to a wide range of tunable mechanical properties besides their biodegradability and biocompatibility [1]. We created a 3D microphysiological brain model by casting photo-polymerized gelatin methacryloyl (GelMA) in a microliter-sized polydimethylsiloxane (PDMS) ring serving for confinement on microscope glass slides (Figure 1a). SH-SY5Y were seeded onto the fibronectin-covered glass at a seeding density of 150,000 cells/cm^2 prior to casting. The cells expressed an elongated, branched, and spreading morphology resembling neurons at day 5 (Figure 1c). Confocal z-stack microscopy endorsed our hypothesis that stiff-to-soft material transitions promoted neuronal migration into the third dimension up to 60 µm distance (Figure 1d). Next, we want to implement this method of cast GelMA in an actuator chip containing a mechanically deformable membrane (Figure 1b). Previously, this brain-on-chip (BoC) enabled the study of primary neuronal cell dynamics either in the 2D or in the 3D culture format using Matrigel as a scaffold [2]. Tailoring of the GelMA Young's modulus will then allow us to control mimicry of ECM stiffness gradients in such a device.

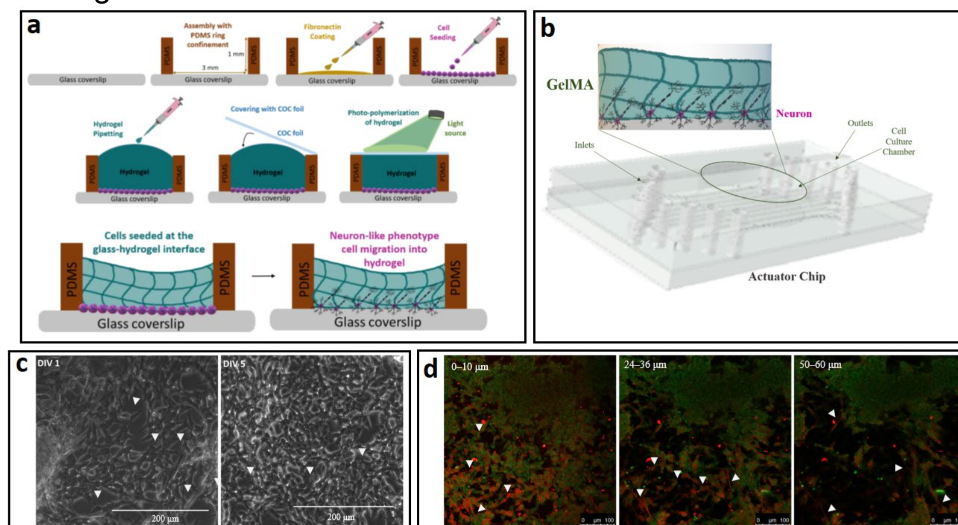

Figure 1: a) GelMA brain model workflow. (b) Schematic of GelMA brain model placed in the actuator chip to yield ECM stiffness gradients in BoC. (c) SH-SY5Y cells imaged in the model on days 1 and 5. White arrows indicate neuron-like phenotypes. (d) Confocal z-stack images of SH-SY5Y cells in the model. Staining: β-Tubulin III (red) and cell nuclei dye (green). Arrows display neuronal migration.

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Microengineered hollow graphene tube systems generate conductive hydrogels with extremely low filler concentration

C. Arndt[1,2], M. Hauck[3], I. Wacker[4], B. Zeller-Plumhoff[5], F. Rasch[3], M. Taale[1,2], A. Shaygan Nia[6], X. Feng[6], R. Adelung[3], R. R. Schröder[4], F. Schütt[3], C. Selhuber-Unkel[2]

[1] *Biocompatible Nanomaterials, Institute for Materials Science, Kiel University, Kiel, Germany*

[2] *Institute for Molecular Systems Engineering (IMSE), Heidelberg University, Heidelberg, Germany*

[3] *Functional Nanomaterials, Institute for Materials Science, Kiel University, Kiel, Germany*

[4] *Cryo Electron Microscopy, Centre for Advanced Materials (CAM), Heidelberg University, Heidelberg, Germany*

[5] *Institute of Metallic Biomaterials, Helmholtz Zentrum Geesthacht, Geesthacht, Germany*

[6] *Department of Chemistry and Food Chemistry, Center for Advancing Electronics Dresden (cfaed), Technische Universität Dresden, Dresden, Germany*

The fabrication of electrically conductive hydrogels is challenging as the introduction of an electrically conductive filler often changes mechanical hydrogel matrix properties. Here, an approach for the preparation of hydrogel composites with outstanding electrical conductivity at extremely low filler loadings (0.34 S m^{-1}, 0.16 vol%) is presented. Exfoliated graphene and polyacrylamide are microengineered to 3D composites such that conductive graphene pathways pervade the hydrogel matrix similar to an artificial nervous system (Figure 1a). This makes it possible to combine both, the exceptional conductivity of exfoliated graphene and the adaptable mechanical properties of polyacrylamide. The demonstrated approach is highly versatile regarding porosity, filler material as well as the hydrogel system. The important difference to other approaches is that we keep the original properties of the matrix, while ensuring conductivity through graphene-coated microchannels (Figure 1b). This novel approach of generating conductive hydrogels is very promising, with particular applications in the fields of bioelectronics and biohybrid robotics.

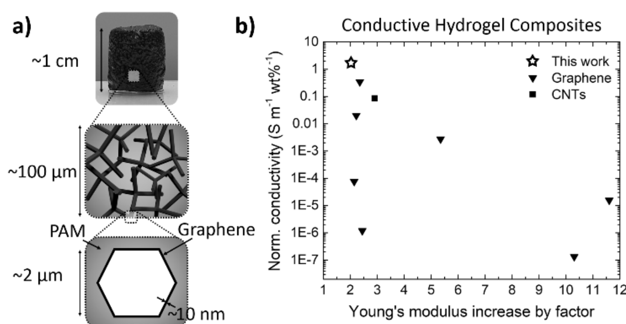

Figure 1 a) Conductive hydrogel sample on different scales. b) Ashby plot showing the weight-percent normalized conductivity as a function of increase in Young’s modulus for different conductive hydrogel systems based on carbon filler material. The star indicates the results for the polyacrylamide-exfoliated graphene composites from this work.

Tuning the nanotopography and functionality of 3D-printed cellular scaffolds through cellulose nanocrystal coatings

Mouhanad Babi[1], Roberto Riesco[2], Louisa Boyer[2], Ayodele Fatona[1], Angelo Accardo[3], Laurent Malaquin[2] and Jose Moran-Mirabal[1].
[1]Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Ontario, Canada
[2] LAAS-CNRS, Université Toulouse III - Paul Sabatier, Toulouse, France
[3]Department of Precision and Microsystems Engineering, Delft University of Technology, Mekelweg 2, Delft

Living cells naturally exist in three-dimensional microenvironments with geometries, topography, stiffness, surface chemistry and biological factors that strongly dictate their behaviour. Often, the microenvironment is based on an organized structure or scaffold that, together with the cells that live within in it, make up living tissue. In order to mimic these systems and understand how the different properties of a scaffold impact the behaviour of a cell, like their adhesion, proliferation or function, we must be able to fabricate cellular microenvironments with tunable properties. In this work, the nanotopography and functionality of cellular scaffolds were modified by coating 3D printable materials (i.e., DS3000 and PEG-DA) with cellulose nanocrystals (CNCs). This general approach was demonstrated on a variety of structures designed to incorporate macro and microscale features and fabricated using photopolymerization 3D printing techniques. Atomic force microscopy was used to characterize the CNC coatings and showed the ability to highly tune their density, and in turn the surface nano-roughening, from isolated nanoparticles to complete and dense surface coverage. The coating of 3D-printed structures with CNCs and the ability to tune their density permitted control over the attachment and morphology of cultured prostate cancer cells. It was also possible to introduce functionalization onto the surface of these scaffolds, either directly by coating them with CNCs grafted with the functionality of interest, or with a more general approach of functionalizing the CNCs after coating using biotin-streptavidin coupling. The ability to carefully tune the nanostructure and functionalization of different 3D-printable materials is a step forward to creating more realistic cellular environments, which are key to understanding their role on a living cell and to the development of artificial tissue.

An Indestructible Tension Probe for Measuring High-Force Mechanical Events in Cells

Rachel L. Bender, **Yuxin Duan, Anna V. Kellner, Hiroaki Ogasawara, Jennifer M. Heemstra, Yonggang Ke, Khalid Salaita**

Emory University, Atlanta, United States

Cells physically interact with their environment and through mechanotransduction, convert cell, integrin, and matrix interactions in their microenvironment into biochemical signaling pathways that guide downstream signaling outcomes and cell fate. Variations in mechanics caused by traction forces with the extracellular matrix have been associated with the development of skeletal muscle, embryogenesis, and metastasis, highlighting the importance of studying this relationship. DNA-based, surface-immobilized tension probes have enabled measurement of cellular forces in piconewton (pN) ranges.[1] However, the structure of DNA tension probes prevents measurement of forces above ~60 pN, and their susceptibility to nuclease degradation restricts their use beyond a few hours.[2,3] To address these limitations, we have developed peptide nucleic acid (PNA) tension probes. PNA is a nucleic acid analogue that can form conventional Watson-Crick-Franklin base pairings with other nucleic acids. It is a highly attractive as a probe for molecular forces due to its superior thermodynamic stability and resistance to degradation by all known enzymes. We have characterized the thermodynamic properties of a library of PNA probes and quantified their biostability in the presence of nucleases and proteases. We have also demonstrated higher mechanical stability of PNA-based tension probes and have successfully used our probes to image the traction forces of NIH 3T3, Human Airway Smooth Muscle (HASM), and MDA-MB-231 cell lines.

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Fabrication of multiplexed curved phospholipid membrane platform

Eider Berganza[1] , **Mirsana P. Ebrahimkutty**[2], **Srivatsan K. Vasantham**[1], **Chunting Zhong**[1], **Alexander Wunsch**[3], **Alexander Navarrete**[3], **Milos Galic**[2], **Michael Hirtz**[1]

[1] *Institute of Nanotechnology (INT-KIT), Eggenstein-Leopoldshafen, Germany*
[2] *University of Münster, Münster, Germany*
[3] *Institut für Mikroverfahrenstechnik (IMVT-KIT), Eggenstein-Leopoldshafen, Germany*

The curvature of lipid membranes plays a key role in many relevant biological processes such as membrane trafficking, vesicular budding or host-virus interactions.[1] In-vitro studies on biomimetic models in the nanoscale are challenging, due to the resolution and high cost of the available techniques. In this work, we propose a simple and low cost platform for curvature sensitive protein screening, prepared through scanning probe lithography (SPL) methods, where lipid bilayer patches of different compositions can be multiplexed onto substrate areas with tailored local curvature[2] The desired curvature is imposed by anchoring nanoparticles of the desired size to the substrate prior to lithography. We demonstrate that a biosensor for positive membrane deformations derived from the BAR domain of Nadrin2 is binds selectively to lipid patches patterned on substrates areas coated with 100 nm nanoparticles. The platform opens up a path to screening applications for protein / curved membrane interaction studies by providing a flexible and easy to prepare substrate with control over lipid composition and membrane curvature.

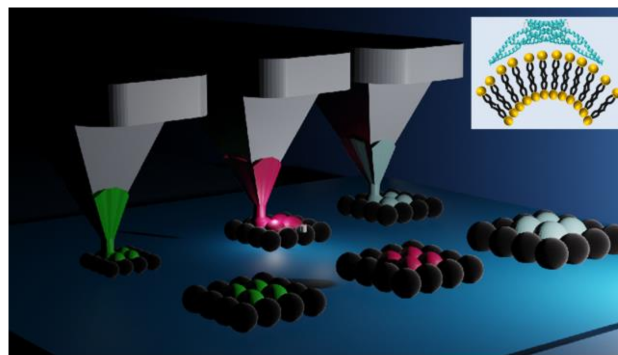

Figure 1. Proposed platform for the multiplexing of lipid patches via Dip-Pen Nanolithography onto locally curved surfaces. In the inset: curvature sensitive protein binding to a tailored curved phospholipid bilayer (adapted with permission from[3]).

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Determination of the actual size of proteins in the live cell plasma membrane

V. Brumovska[1], G. Fülöp[1], G. J. Schütz[1], E. Sevcsik[1]

[1] *Institute of Applied Physics, TU Wien, Wiedner Hauptstraße 8-10, 1060 Vienna, Austria*

It is well established that lipids and proteins are not just independent components of the plasma membrane of eukaryotic cells but that their arrangement, dynamics and function are interdependent. Besides specific lipid-protein interaction, transmembrane proteins are thought to bind a shell of annular lipids, which are more or less tightly associated with the proteins. Furthermore, highly ordered nanoscopic membrane domains have been proposed to act to compartmentalize proteins and their interactions, but have thus far not been directly observed.

Here, we use a combination of single molecule tracking and protein micropatterning to examine these interactions directly in the plasma membrane of living cells. In our experimental approach, different proteins of interest are immobilized within defined patterns in the plasma membrane, where they act as steric obstacles to the diffusion of lipid tracers and thus locally decrease their mobility. In the presence of lipid-protein interactions of any type, lipid mobility will be even further decreased within protein patterns. We used different types of proteins for patterning – single- and multi-spanning transmembrane proteins, a multi-subunit protein and a GPI-anchored protein. For all proteins we found that lipid tracer diffusion within protein patterns was slowed down only by steric hindrance since the size of the protein “sensed” by the diffusing lipid corresponded well with the size estimated from the protein crystal structure. These findings indicate that none of the examined proteins influence their membrane environment beyond their physical size, neither via tightly associated annular lipids nor via more ordered membrane domains.

Dynamics of stem cell migration and condensation on nanopatterned adhesive ligands

I. Casanellas[1,2,3], A. Lagunas[3,1,*], Y. Vida[4,5], E. Pérez-Inestrosa[4,5], J. A. Andrades[3,6], J. Becerra[3,5,6] and J. Samitier[1,2,3]

[1]Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain
[2]Department of Electronics and Biomedical Engineering, University of Barcelona (UB), Barcelona, Spain
[3]Biomedical Research Networking Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), Madrid, Spain
[4]Departament of Organic Chemistry, University of Málaga-IBIMA, Málaga, Spain
[5]Andausian Centre for Nanomedicine and Biotechnology-BIONAND, Málaga, Spain
[6]Department of Cell Biology, Genetics and Physiology, University of Málaga-IBIMA, Málaga, Spain
** Corresponding author: alagunas@ibecbarcelona.eu*

Mesenchymal cell condensation is a prevalent morphogenetic transition in which mesenchymal stem cells (MSCs) gather to form three-dimensional structures that set the architectural foundations of tissues such as cartilage. This process is dependent on the ability of cells to migrate towards condensation centers and establish the initial cell-cell contacts.

We have previously shown that dendrimer-based RGD nanopatterned substrates can be tuned to modulate MSC adhesion, differentiation, mechanotransduction and intercellular communication during cartilage formation (1-3); here we use RGD nanopatterns to study the influence of local ligand density on MSC migration dynamics in the process. We live imaged the first 40 hours of condensation to quantify cell displacement, speed and directionality, as well as cell-cell collisions; and we pharmacologically blocked cell-substrate and cell-cell interactions to investigate their role in the regulation of single and collective cell migration.

We find that nanopatterns of intermediate ligand density lead to faster movement and more cell-cell collisions, facilitating the condensation process, whereas high homogenous adherence impedes it by making cells slower and highly directional. Migration of whole cell condensates also depends on ligand density. Blocking certain sets of interactions differently affects single cell and condensate migration.

These findings provide insight on the regulation of single and collective stem cell behavior through nanoscale environmental cues during tissue development. They contribute to the design of nanobiomaterials for the regeneration of tissues such as cartilage, among other applications that require precise control over cell movement.

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Imaging membrane deformation during nanoparticle uptake at the ventral side

J. Christian[1], C. Funaya[2], U. Schwarz[3,4], E.A. Cavalcanti-Adam[1]

[1]*Max Planck Institute for Medical Research, Heidelberg, Germany*
[2] *Electron Microscopy Core Facility Universität Heidelberg, Heidelberg, Germany*
[3] *Bioquant Center, Heidelberg, Germany*
[4] *Institute of Theoretical Physics Universität Heidelberg, Heidelberg, Germany*

Membrane deformation is crucial for endocytosis[1]. Imaging membrane deformation at the ventral side of an adherent cell during nanoparticle uptake with unprecedented nanoscale resolution has been achieved by combining ultramicrotomy and electron microscopy[2]. So far, low throughput is the main challenge despite arduous efforts invested in preparing samples and acquiring images.

In this work, we reproducibly control cell shape and the adhesion location using the combination of photolithography and surface chemistry. Furthermore, we apply surface functionalization strategies to control the immobilization of 300 nm gold nanoparticles on surfaces. On 100 μm cross-bow-shaped fibroblast cells, we observed that gold-particle uptake at the ventral side is adapted more towards the central part of the cell, while at the edge, cells most likely roll particles to the dorsal side.

By controlling parameters such as the adhesion location and cell shape, the sample preparations for EM imaging of membrane deformation during particle uptake can be made easier together with an increasing success rate. It helps to understand further the contribution of different cell structures in the stabilization of endocytic complexes.

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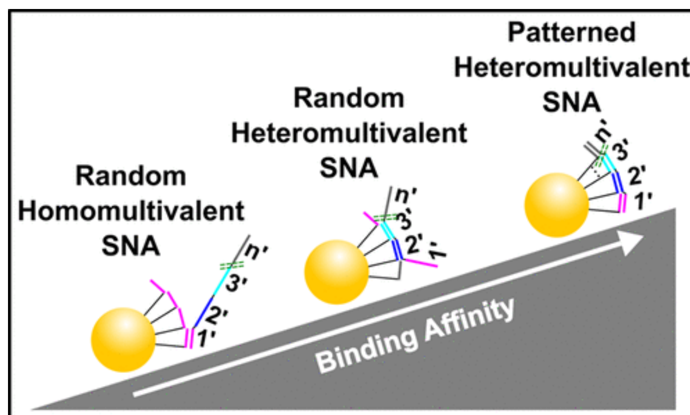
Engineering DNA-Functionalized Nanostructures to Bind Nucleic Acid Targets Heteromultivalently with Enhanced Avidity

Brendan R. Deal, Rong Ma, Victor Pui-Yan Ma, Hanquan Su, James T. Kindt, and Khalid Salaita

Department of Chemistry, Emory University, Atlanta, Georgia, United States



Improving the affinity of nucleic acids to their complements is an important goal for many fields spanning from genomics to antisense therapy and diagnostics. One potential approach to achieving this goal is to use multivalent binding, which often boosts the affinity between ligands and receptors, as exemplified by virus–cell binding and antibody–antigen interactions. Herein, we investigate the binding of heteromultivalent DNA–nanoparticle conjugates, where multiple unique oligonucleotides displayed on a nanoparticle form a multivalent complex with a long DNA target containing the complementary sequences. By developing a strategy to spatially pattern oligonucleotides on a nanoparticle, we demonstrate that the molecular organization of heteromultivalent nanostructures is critical for effective binding; patterned particles have a ~23 order-of-magnitude improvement in affinity compared to chemically identical particles patterned incorrectly. We envision that nanostructures presenting spatially patterned heteromultivalent DNA will offer important biomedical applications given the utility of DNA-functionalized nanostructures in diagnostics and therapeutics.

Biophysical cues affect neural network dynamics

Y. Demircan Yalcin[1], J.-P. Frimat[1, 2], and R. Luttge[1]

[1]*Neuro-Nanoscale Engineering, Eindhoven University of Technology, Eindhoven, Netherlands*

[2]*Current address: Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands*

This study presents neural network dynamics of human induced pluripotent stem cell (hiPSC)-derived neurons cultured on multielectrode arrays (MEAs) with and without Matrigel. Elasticity is an important biophysical cue in the development of neuronal networks and is provided by the complexity of the extracellular matrix (ECM) [1]. Previously, it was assumed that the Matrigel on top of primary cortical neurons can create a favorable ECM mimicking milieu to obtain better connectivity between neurons [2]. Xie *et al.* used calcium imaging to observe cell culture with Matrigel but it is challenging to make quantitative conclusions. By using MEA technology, here, we quantitatively analyzed spike, burst, and network burst (NB) rate of cells, and found differences in neural network behavior as a response to the addition of Matrigel. Differentiation procedure was applied by following the steps in [3]. Measurements were carried out via 2100MEA system (Multi Channel Systems GmbH, Germany) by using 120 electrodes MEA. After 3 weeks in cell culture, 2D to 3D transitions can occur under 3D culture conditions and network dynamics change due to this transition [4]. Therefore, the data collected at 8, 14, and 21 days-*in vitro* (DIV) were compared to eliminate this influence. Table 1 presents the results showing significant increase in spike and burst rates at each DIV while NB rates were similar. To make further interpretation about the introduction of ECM mimicking milieu atop of the 2D cell layer on MEA substrates, these results will be repeated in follow up experiments also with different types of hydrogels.

Table 1: Spike, burst, and NB rates (per minute) of 2D hiPSCs-derived neuronal cell cultures without Matrigel and having Matrigel on top of them.

	8DIV			14DIV			21DIV		
	Spike	Burst	NB	Spike	Burst	NB	Spike	Burst	NB
2D without Matrigel	1690	14	1	2688	13	0.5	5448	28	1,5
2D with Matrigel	4073	25	1,3	9408	66	0,75	9524	70	1,4

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Democratizing Mechanobiology with Mechanically-Triggered Hybridization Chain Reactions

Yuxin Duan[1], Roxanne Glazier[2], Alisina Bazrafshan[1], Yuesong Hu[1], Brian Petrich[4], Yonggang Ke[2], Khalid Salaita[1,2]

[1] *Department of Chemistry, Emory University, Atlanta, USA.*
[2] *Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, USA.*
[3] *Department of Pediatrics, Emory University, Atlanta, USA.*

The vast majority of cells have evolved the ability to transmit molecular piconewton forces to their cell surface receptors to mediate a range of processes such as cell migration, development, immune recognition and coagulation. Over the past decade, a suite of molecular tension sensors have been developed by our lab and others to quantify such forces, and these probes have advanced our understanding of mechanotransduction, which is the interconversion of physical forces and biochemical signals. A major challenge in this area is the sparse number of mechanical events generated by cells. Accordingly, molecular tension signal is typically quantified using high resolution fluorescence microscopy, which hinders the potential for widespread adoption and screening applications. To address this need, we report the development a mechanically-triggered hybridization chain reaction (HCR) which allows the chemical amplification of mechanical events with DNA nanotechnology. The amplification is triggered only if a cryptic nucleic acid HCR initiator is revealed when a cell surface receptor mechanically denatures a blocking oligonucleotide strand. The mechano-HCR design allows the propagation of nucleic acid polymers at the site of mechanical events exceeding 12pN. This amplification leads to a 10-fold enhancement in S/N when compared to direct measurement of duplex rupture. Importantly, the enhanced signal due to mechano-HCR allows for direct readout of cellular traction forces using a conventional fluorescence plate reader. We leverage this capability and demonstrate measurements of drug mechano-IC50 (concentration of a drug that leads to 50% inhibition of receptor force), for aspirin, ROCK inhibitor, and eptifibatide. The mechano-IC50 values match that of literature reported IC50's, validating the rigor of this readout for conducting potential screens. Given that microscopic measurements of cell traction forces have demonstrated clinical potential, mechano-HCR may complement such assays by offering a convenient route for molecular screening of mechanical phenotypes for applications that may span clinical screening to drug discovery.

Biomolecule micropatterning on different polymeric substrates and its applicability for quantitative fluorescence microscopy

R. Hager[1], C. Forsich[1], D. Heim[1], J. Weghuber[1] and P. Lanzerstorfer[1]

[1] *University of Applied Sciences Upper Austria, Wels, Austria*

The absence of functional groups in many polymeric materials does not allow for the immobilization of biomolecules onto these substrates by means of common surface chemistry. However, polymeric materials play an emerging role in the development of new biomedical and biosensing devices. Here, we present an effective method for the selective functionalization of different polymeric substrates with biomolecules and give an overview of their suitability for fluorescence microscopy. For the transfer of biomolecules onto the functionalized polymeric surface, microcontact printing (µCP) is used. We have already demonstrated other approaches, e.g. photolithography, for the fabrication of micropatterns on cyclic olefin polymers (COPs) [1]. However, the implementation of photolithographic approaches for the fabrication of microstructured surfaces is expensive and labor-intensive compared to µCP. We evaluate the suitability of different polymers for biomolecule immobilization via contact angle measurement, scanning electron microscopy (SEM) and fluorescence microscopy. Furthermore, micropatterned polymeric substrates were tested for their applicability in live cell assays via total internal reflection fluorescence (TIRF) microscopy.

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Analysis of FGFR3 signaling strength using Micropatterning

Ingrid Hartl[1]*, Veronika Brumovska[2]*, Yasmin Striedner[1], Atena Yasari[1], Gerhard Schütz[2], Eva Sevcisk[2], Irene Tiemann-Boege[1]

[1] Institute of Biophysics, Johannes Kepler University, Linz, Austria
[2] Institute of Applied Physics, Technical University, Vienna, Austria
**equal contribution*

Fibroblast growth factor receptors (FGFRs) are a class of receptor tyrosine kinase receptors that are activated upon ligand-induced receptor dimerization, which triggers kinase domain trans-autophosphorylation followed by a multistep intracellular signaling cascade. Tight regulation of the FGFR signaling is paramount in different developmental stages, as well as for tissue crosstalk. Impaired or aberrant FGFR signaling has been shown to be the cause for various genetic disorders or pathologic conditions, as for example cancer. [1,2] Presently, Western Blotting is considered the golden standard to quantify FGFR signaling assessed by the phosphorylation of the receptor kinase domain. Yet, this technique is very labor-intensive, time-consuming, and renders only semi-quantitative data at best. [3] Here we present a new method to measure the activation of FGFR3 by the receptor's recruitment of down-stream signaling adaptor proteins (e.g. GRB2) using the microscope-based technique known as micropatterning. In this method, HeLa cells are co-transfected with two expression constructs: mGFP labeled FGFR3 and mScarlet-I labeled GRB2. The recruitment of the adaptor protein to the phosphorylated receptor is assessed by the co-localization of micropatterns of both fluorophores. We tested the activation of FGFR3 upon ligand binding (fgf1 and fgf2) in the wildtype (WT) form and negative and positive controls, as well as in different FGFR3 mutants associated with congenital disorders. Our data show that the addition of ligands leads to increased GRB2 recruitment to WT FGFR3, with fgf1 having a stronger effect than fgf2. We also show that FGFR3 mutations result in an already pre-activated receptor that can be further triggered by ligand addition. However, some pre-activated mutant receptors are not responsive to the ligand. Interestingly, some of these mutants have an unexpectedly low activation state compared to Western Blotting measurements. These discrepancies might be related to signaling events of FGFR3 happening at the plasma membrane captured only with micropatterning versus bulk activation measured in whole cell lysates by Western Blotting.

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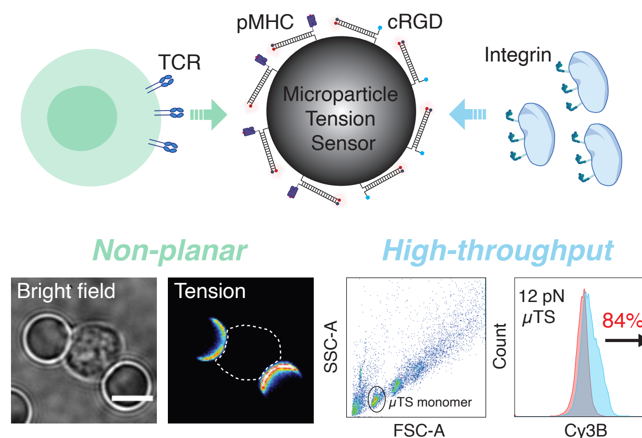
Microparticle molecular tension sensors for mapping cell mechanics in non-planar geometries and for high-throughput quantification

Y. Hu,[1] V. P-Y. Ma,[1] R. Ma,[1] W. Chen,[2] Y. Duan,[1] R. Glazier,[3] B. Petrich,[2] R. Li[2] and K. Salaita[1,3]

[1]*Department of Chemistry, Emory University, Atlanta, GA, USA*
[2] *Department of Pediatrics, Emory University, Atlanta, GA, USA*
[3] *Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA*

Mechanotransduction, the interplay between physical and chemical signaling, plays vital roles in many biological processes ranging from cell differentiation to metastasis. The state-of-the-art techniques to quantify cell forces employ deformable polymer films or molecular probes tethered to glass substrates. A fundamental challenge in this field is the planar geometry of the force-sensing substrate, which contrasts against the more physiological cell-cell and cell-ECM interactions that mostly occur in non-planar geometries. A second challenge is the low throughput of microscopy readout which limits the application of current assays in fundamental and clinical research. We address these challenges by developing a DNA-based microparticle tension sensor (μTS), which features a spherical surface and thus allows for investigation of mechanical events at curved interfaces or within groups of cells in suspension. Importantly, the micron-scale of μTS enables flow cytometry readout, which is rapid and high throughput. To demonstrate the scope of μTS, we applied the method to map and measure T-cell receptor (TCR) forces and platelet integrin forces at 12 and 56 pN thresholds. Furthermore, we quantified the inhibition efficiency of two anti-platelet drugs providing a proof-of-concept demonstration of μTS to screen drugs that modulate cellular mechanics.



Nanostructured surfaces via self-assembly of DNA-spider silk conjugates

M. Humenik, **A. Molina, T. Preiß and T. Scheibel**

University Bayreuth, Faculty of Engineering Science, Department of Biomaterials, Bayreuth, Germany

We established DNA-spider silk conjugates using “click” coupling of the recombinant protein eADF4(C16) with short DNA strands to create hybrid materials. Whereas the spider silk moiety enabled self-assembly into nanofibrils controlled by phosphate ions in aqueous buffers, the DNA part enabled DNA specific fibril labeling [1] or DNA-hybridization based self-organization of the fibrils into hierarchically ordered nano-ribbons and microscopic rafts [2]. In our recent work, we applied sequence specific DNA hybridization for the immobilization of DNA-spider silk conjugates on complementary modified surfaces. Addressing of the conjugates onto predestined spots was achieved using micro-contact printing. Addition of the unmodified protein and phosphate buffer triggered localized protein nucleation and fibril self-assembly on the surface resulting in nanofibril-based patterns with a submicrometer resolution [3]. Further, we conjugated the spider silk protein with DNA-aptamers, which specifically bind to a selected enzyme. The spider silk moiety did not disturb the aptamer-enzyme binding and enabled immobilization of the aptamer via nanofibril self-assembly on surfaces. The network of fibrils revealed properties of immobilized nanohydrogels which swelling enabled high enzyme binding capacity. Moreover, a specific switch in the secondary structure of the immobilized DNAs allowed fast release of the active enzyme [4].

In summary, we have demonstrated that the localization of nucleation and self-assembly of spider silk cross-beta nanofibrils on surfaces is possible. This approach is compatible with soft lithography allowing complex patterns of fibrils in a submicrometer range as well as incorporation of ligand-binding aptamers for specific immobilization and release of active enzymes. The DNA-functionalized nanohydrogels represent nanostructured surfaces with potential for controlled cell binding studies.

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Micropatterned arrays of peptide-receptive MHC class I proteins

Nouria Jantz-Naeem[1], Cindy Dirscherl[1], Sebastian Springer[1]

[1] *Jacobs University Bremen, Bremen, Germany*

Micropatterned surfaces are promising tools for screening applications. For the screening of cytotoxic T cells, arrays of major histocompatibility complex (MHC) class I proteins have been suggested. Current approaches generally describe the spotting of individual MHC class I molecules complexed with antigenic peptide (pMHC I) on surfaces. Instead of printing the pMHC I complex, we demonstrate the fabrication of peptide-free MHC class I arrays which can be loaded individually with test peptides. We use microcontact printing to immobilize empty MHC class I molecules in geometric structures on glass surfaces. We demonstrate that the printed MHC class I molecules are fully functional and can be specifically loaded with antigenic peptides. Additional imaging techniques have also shown promise for potential array readout. The ulterior goal is to develop a ready-to-use microfluidic MHC class I array which can be loaded with candidate peptide and serve as point-of-care T cell screen of tumor patients.

A visual immunoprecipitation assay for live-cell profiling of cytosolic protein complexes on micropatterned substrates

R. Hager[1], U. Müller[1], N. Ollinger[1], J. Weghuber[1], P. Lanzerstorfer[1]

[1]*University of Applied Sciences Upper Austria, Wels, Austria*

Analysis of protein-protein interactions in living cells by protein micropatterning is currently limited to the spatial arrangement of transmembrane proteins ('bait', e.g. receptor) and their corresponding downstream adaptor molecules ('prey'). Here we present a method for visual immunoprecipitation of cytosolic protein complexes by use of an artificial transmembrane bait construct in combination with micropatterned antibody arrays on cyclic olefin polymer (COP) substrates. The method was used to characterize initial signalling complexes of the Ras-Raf-MEK and PI3K-Akt pathway downstream the epidermal growth factor receptor (EGFR) on a single cell level. We found prominent differences in EGFR-mediated cytosolic protein complex formation. Interaction dynamics of cytosolic effector proteins were unambiguously quantified by fluorescence recovery after photobleaching as well as by the use of Grb2-related protein domain inhibitors. Furthermore, implementation of specific Grb2-mutants confirmed the role of SH2/SH3 domains in controlling binding events with a variety of adaptor molecules. In general, the assay sheds light on the importance of in-depth characterization of cytosolic protein-protein interactions as regulatory mechanisms for coordinated cellular downstream signalling.

It's quantum biology, Jim, but not as we know it

Graham Leggett, Department of Chemistry, University of Sheffield, UK

Physicists have long been interested in the idea that there may be biological processes that require a specifically quantum-mechanical explanation. However, this is an idea that is shrouded in controversy. We have addressed the quantum biology question from a different perspective: rather than asking whether biology can be controlled by quantum processes, we have shown that biology can be used to control quantum processes. We have found that localised surface plasmon resonances associated with gold nanostructure arrays are strongly coupled to excitons in light-harvesting complexes from plants and bacteria, leading to the formation of hybrid states called plexcitons that mix the properties of light and matter. The properties of these macroscopically extended excited states are manipulated via control of the protein structure and pigment complement. Plexcitons involve collective phenomena: the plasmon model couples to an ensemble of excitons, facilitating coherent, ultra-fast exchange of energy via the light field across distances too long for excitonic coupling through space. This allows the creation of bespoke transitions, not seen under weak coupling and creates the attractive prospect of designing programmable materials for applications in molecular photonics.

1D nanopatterned integrin ligand surface to evaluate stick-slip dynamics

V. Levario-Diaz, A. Fink, W. Feng and E. A. Cavalcanti-Adam

Max Planck Institute for Medical Research, Heidelberg, Germany

Cell-extracellular matrix (ECM) adhesion modulated by integrin receptors is a highly regulated process involved in many vital cellular functions such as motility, proliferation and survival.[1] The influence of integrin lateral clustering in driving the coordination of cell front and back dynamics during cell migration remains unsolved. For this purpose, defined 1D micro-nanopatterned stripes based on block-copolymer micelle nanolithography technique with biofunctionalized gold nanoparticles with the integrin-specific RGD (arginine-glycine-aspartate) motif were fabricated.[2,3] The 10 um-wide stripes consist of a quasi-perfect hexagonal arrangement of gold particles, with a mean diameter of 8 nm that serve as an anchoring site for a single integrin heterodimer. The gold nanoparticles were placed with a spacing of 50, 80 and 100 nm to regulate integrin clustering and focal adhesion dynamics, which affect the cytoskeleton structures that regulate the front and back cellular motion. By employing time-lapse microscopy and immunostaining, we propose that the speed and coordination of stick-slip movement and the migratory behavior of fibroblasts changes according to the nanoscale spacing of adhesion sites.

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Whether is impact of the low concentration of the $CaCO_3$ nanoparticles embedded in non-fouling temperature-responsive POEGMA coatings on different cell lines?

O. Lishchynskyi[1], Y. Stetsyshyn[1], J. Raczkowska[2], K. Awsiuk[2], A. Abalymov[3], A. Skirtach[3], Y. Shybanova[1], A. Budkowski[2]

[1]*Lviv Polytechnic National University, Lviv, Ukraine*
[2]*Smoluchowski Institute of Physics, Jagiellonian University, Kraków, Poland*
[3]*Department of Biotechnology, Ghent University, Ghent, Belgium*

In the present work, we have successfully prepared and characterized novel nanocomposite material exhibiting temperature-dependent surface wettability changes, based on grafted brush coatings of non-fouling POEGMA with the embedded $CaCO_3$ NanoParticles. Grafted polymer brushes attached to the glass surface were prepared in a three-step process using ATRP polymerization. Subsequently, uniform $CaCO_3$ NPs embedded in POEGMA grafted brush-coatings were synthesized using biomineralized precipitation from solutions of $CaCl_2$ and Na_2CO_3.

An impact of the low concentration of the embedded $CaCO_3$ NPs on cell adhesion and growth depends strongly on the type of studied cell line: keratinocytes (HaCaT), melanoma (WM35) and osteoblastic (MC3T3-e1). Based on the temperature responsive properties of grafted brush coatings and $CaCO_3$ NPs acting as biologically active substrate, we hope that our research will lead to a new platform for tissue engineering with modified growth of the cells due to the release of biologically active substance from $CaCO_3$ NPs and ability to detach the cells in a controlled manner using temperature-induced changes of the brush.

Keywords: grafted coatings, nanoparticles, cells, poly(di(ethylene glycol)methyl ether methacrylate), cell adhesion, HaCaT, WM35, MC3T3-e1.

Force-distance curve-based AFM and single cell approaches to probe ligand-receptor interactions and mechanics

C. Lo Giudice[1], **D. Alsteens**[2], **E.A. Cavalcanti-Adam**[1]

[1]Max Planck Institute for Medical Research, Heidelberg, Germany
[2] Université catholique de Louvain, Louvain-La-Neuve, Belgium

The interplay between chemical and mechanical stimuli plays a key role in regulating cells and tissue homeostasis and its unbalance may quickly lead to malignant transformations [1,2]. In this respect, cell surface receptors are key components as they control and mediate cell communication and signalling. Elucidating the molecular details of ligand-binding to cell surface receptors and the associated signal transmission initiating intracellular signalling is therefore necessary for a thorough understanding and control of cellular processes and for rational drug design [3,4].
In the last decades, sophisticated nanotechnology and biophysical tools have been developed to tackle biological problems down to the single-cell and single-molecule level. With its piconewton force sensitivity and (sub)nanometer spatial resolution, together with the capability of operating in liquid environment and at physiological temperature, force-distance curve-based atomic force microscopy (FD-based AFM) has proven to be one of the most powerful tools to image and quantify receptor-ligand bonds at the single molecule level in conditions mimicking their native environment, as well as for the mechanical mapping and manipulation of biomolecules and mammalian cells [5]. Here we present several applications of FD-based AFM and relevant single cell techniques in the field of cell- and mechanobiology. Primarily, we show the *in situ* probing of glucagon-binding to individual glucagon receptors coupled with the dynamical mechanical mapping of its transition to the active conformer [6]. Applications in the area of nanobiointeractions, endocytosis and crosstalk between growth factor receptors and cell adhesion machinery will also be presented.

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Membrane curvature regulates the spatial distribution of the glycocalyx proteins

Chih-Hao Lu[a], Kayvon Pedram[a], Taylor Jones IV[a], Ching-Ting Tsai[a], Xiao Li[a], Carolyn R. Bertozzi[a, b], and Bianxiao Cui[a,*]

[a]Department of Chemistry, Stanford University, Stanford, CA, USA
[b]Howard Hughes Medical Institute, USA

The glycocalyx is a shell of heavily glycosylated proteins and lipids distributed on the surface of plasma membranes of nearly all cell types. Large and bulky transmembrane glycoproteins have been shown to impact the membrane shape. For example, upregulation of the large glycoprotein MUC1 is believed to be responsible for the extensive surface protrusions of cancer cells. Due to the physical attachment of the glycocalyx to cell membranes, the extracellular bulky domain of glycopolymers such as MUC1 may be able to sense membrane curvatures and redistribute on the plasma membrane. However, little is known about how the glycocalyx responds to different membrane curvatures, and whether the glycocalyx impacts cell-nanomaterial interaction. Here, we took advantage of a multi-disciplinary approach to address this knowledge gap at the nano-bio interface. With the aid of nanoscale manipulation and BAR-family proteins, we were able to generate well-defined membrane curvatures physically or biologically to investigate the curvature sensitivity and preference of an ubiquitous glycocalyx protein-MUC1. Our results demonstrate that 1) MUC1 was excluded from the nanostructure-membrane interfaces with positive curvatures. Moreover, this phenomenon is dependent on the number of N-terminal tandem repeats of MUC1. 2) MUC1 preferentially accumulated at both physically- and biologically-induced negatively-curved membranes. Such curvature preference of mucin-like glycopolymer was conserved in vitro. 3) The curvature sensitivity and preference of MUC1 rely on its ectodomain and O-linked glycosylation state. 4) We also discovered that MUC1 strongly impeded cell adhesion on the densely-coated nanostructured surfaces. These observations implicate the potential roles of the glycocalyx in many vital cellular processes involving membrane deformations, and point out the impacts of bulky glycocalyx on the cell-nanomaterial interaction.

Ensemble measurement of TCR-pMHC force lifetime by DNA hybridization kinetics

Rong Ma[1], Aaron Blanchard[1,2], Brendan R Deal[1], Yuesong Hu[1], Anna V Kellner[1,2], Khalid Salaita[1,2]

[1]*Emory University, Atlanta, USA*
[2] *Georgia Institute of Technology, Atlanta, USA*

Crawling T cells transmit mechanical forces to TCR-pMHC bonds that form at the physical contacts between T cells and target cells. A significant body of literature has confirmed that such forces modulate TCR triggering and T cell function. Single molecule force spectroscopy studies have shown that the lifetime of the TCR-pMHC complex under mechanical force is an important parameter that predicts triggering of a T cell response. These single-molecule measurements have moved the field closer toward better understanding the exquisite sensitivity and specificity of the TCR triggering, but nonetheless a problem inherent in all these measurements is that the experimenter applies the external force, and the actual duration of the TCR-pMHC force remains unknown. Moreover, single molecule techniques are unable to capture the complexity of TCR oligomerization and the role of co-receptors in modulating the force lifetimes. To address these issues, we present the concept of utilizing DNA hybridization kinetics as a benchmark to measure the force lifetime of TCR-pMHC interactions. We take advantage of the DNA hairpin tension probe (1) and the mechanical information storage approach (2) where a complementary "lock" oligonucleotide selectively hybridizes to a cryptic binding site on the DNA hairpin only when it is mechanically revealed by TCR-pMHC interaction. For hybridization to the cryptic site to occur at appreciable rates, hybridization must be faster than the rate of TCR-pMHC force termination. By titrating the concentration of the lock, the hybridization rate at different concentrations can serve as benchmarks for inferring the force lifetime. As a proof of concept, we applied this method to OT-1 T cells and measured the force lifetimes of OT-1 TCR to its cognate antigen, and further analyzed the effect of actin networks and co-receptor engagement on the TCR-pMHC force lifetimes. This method allows for the measurement of the force lifetimes for TCR-pMHC interaction formed at cell-substrate junctions and will aid in improving our understanding of the molecular basis for T cell triggering.

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Study of Interaction of nanomaterials with cells and tissues using *Drosophila melanogaster*

Monalisa Mishra*

Neural Developmental Biology lab, Department of Life Science, National Institute of Technology, Rourkela, Odisha, India-769008

**Corresponding author: mishramo@nitrkl.ac.in*

Nanotechnology is an emerging and rapidly developing technology in the 21st century. Nanomaterials are the chemical form of materials that are at the range of less than 100nm. It is classified as various forms including metal, metal oxide, carbon, silica, semiconductors etc. Nanomaterials are used in various industries including cosmetics, food, medicines, industries, agriculture etc. Alongwith its wide application toxicity is also reported from studies of various model organsim. The toxicity includes: cytotoxicity, genotoxicity and teratogenicity in various model organsims. The current study summerises various reactive oxygen species (ROS) mediated mechanisms that can induce toxicity within the fly[(1).] Nanoparticles are well known to generate ROS once entered into the living system. ROS includes hydroxyl ions, peroxide ions, superoxide anions, singlet oxygen, hypochlorous acids etc. An elevated amount of ROS can damage the cells by various mechanisms. The excess amount of ROS level within the body is futher regulated by a set of antioxidant enzymes like peroxidase, glutathione peroxidase and catalase[(2).] If the antioxidant enzymes cannot nullify the elevated ROS level than DNA damage, cell damage, cytotoxicity, apoptosis and uncontrolled cell regulations occur resulting in abnormal physiological conditions. Herewith we are reporting various morphological and physiological defects caused after nanoparticle treatment as a function of redox imbalance.

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Clinically Relevant Bacteria Outer Membrane Models for Antibiotic Screening Applications

Z Mohamed[1], **JH Shin**[2], **S Ghosh**[3], **F Pinnock**[3], **S Farnush Bint E Naser**[3], **T Dörr**[2], **S Daniel**[1,3]

[1] *Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY, USA*

[2] *Weill Institute for Cell and Molecular Biology, Cornell, University, Ithaca, NY, USA*

[3]*Robert F. Smith School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, USA*

Antibiotic resistance is a growing global health concern that has been increasing in prevalence in the past few decades. In Gram-negative bacteria, changes in membrane properties such as surface charge, length of membrane components such as lipopolysaccharide (LPS), and in physical properties of the membrane such as domain clustering, can alter responses to antibiotics and mediate resistance. Model membranes have been used to mimic bacteria membrane but do not always capture all the components of bacteria membranes such as proteins, LPS, and diverse lipid composition. We developed a substrate-supported membrane platform using outer membrane vesicles (OMVs) from clinically relevant strains to characterize biophysical membrane properties and investigate the interactions between bacteria membrane and antibacterial compounds. OMVs are naturally shed by Gram-negative bacteria from the outer membrane and recapitulate composition and physical properties of the outer membrane. Using clinically relevant isolates, we visualized the rupture and formation of fluid bilayers by monitoring the spread of a membrane-intercalating fluorescent dye, octadecyl rhodamine B. We then confirmed two-dimensional fluidity and measured diffusion coefficients using fluorescence recovery after photobleaching (FRAP) of these bilayers. Using total internal reflection fluorescence microscopy (TIRFM), we confirmed the presence of outer membrane material, including lipid A of LPS and outer membrane proteins, via antibody binding and amine conjugation. To confirm these OMV bilayers could recapitulate known membrane interaction patterns with specific antibiotic compounds, we used fluorescently labeled polymyxin B and bacitracin to show expected membrane behavior. Taken together, this work promotes OMV bilayers as a useful platform for studying clinical Gram-negative isolates and antibiotic membrane interactions while retaining membrane components found in native bacteria membrane. Such platforms could be used to investigate underlying biophysical differences in strains with varying membrane resistances or for the screening of new membrane-active antibiotics.

Mechanical Regulation of the Cytotoxic Activity of Natural Killer Cells

Lital Mordechay[1,2], Guillaume Le Saux[1,2], Avishay Edri[3], Uzi Hadad[2], Angel Porgador[3], Mark Schvartzman[1,2]

[1]Department of Materials Engineering, [2]Ilse Katz Institute for Nanoscale Science & Technology, [3]The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel.

Mechanosensing has been recently explored for T cells and B cells and is believed to be a part of their activation mechanism.[1–4] Here, we explored the mechanosensing of the third type of lymphocytes – Natural Killer (NK) cells, by showing that they modulate their immune activity in response to changes in the stiffness of a stimulating surface. Interestingly, we found that this immune response is bell-shaped, and peaks for the stiffness of a few hundreds of kPa.[5] This bell-shaped behavior was observed only for surfaces functionalized with the activating ligand MHC class I polypeptide-related sequence A (MICA), but not for the control surfaces, lacking immunoactive functionalities. We found that stiffness does not affect uniformly on all the cells but increases the size of a little group of extra-active cells, which in turn contributes to the overall activation effect of the entire cell population. We further imaged the clustering of costimulatory adapter protein DAP10 on the NK cell membrane and found that it shows the same bell-shaped dependence to surface stiffness. Our findings reveal what seems to be "the tip of the iceberg" of mechanosensation of NK cells, and provide an important insight into the mechanism of their immune signaling.

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Implementation of DNA surface technology on large-area micropatterned substrates for interaction analysis in live cells

U. Müller[1], K. Gordiyenko[2], J. Weghuber[1], C. M. Niemeyer[2], P. Lanzerstorfer[1]

[1] *University of Applied Sciences Upper Austria, Wels, Austria*
[2] *Karlsruhe Institute of Technology, Institute for Biological Interfaces (IBG-1), Eggenstein-Leopoldshafen, Germany*

Protein micropatterning has become a functional tool for the spatial arrangement of transmembrane and intracellular proteins in living cells. Among the numerous methods for surface patterning of cover slips and microscopy slides, soft lithography via microcontact printing (µCP) is one of the most convenient and widely used methods. Here, we present an extension of this method to functionalize the glass bottom of 96-well plates using large-area perfluoropolyether (PFPE)- and polydimethylsiloxane (PDMS)-based elastomeric stamps. [1] The resulting ready-to-use multi-well plate can be functionalized in a highly modular manner to contain arbitrary micron-scale patterns of various protein architectures that can be used for high-content experimental analysis of protein-protein interactions in live cells. Importantly, multiscale origami structures as interfaces for cells (MOSAIC) [2] were implemented on those microstructured substrates. Within this regard, DNA oligonucleotides represent programmable and highly modular linker systems that can even provide nanostructure scaffolds for ligand attachment. The resulting surface-patterning approach represents a simple and robust procedure for substrate patterning that also allows for large substrate functionalization with high reproducibility. Furthermore, the MOSAIC technology enables specific and highly defined spatial arrangements of proteins on a nanometer length scale which allows for the study of nanoscale effects, particularly in the early stages of cell signaling. Moreover, we found that the use of DNA origami structures is directly associated with reduced unspecific bait and prey protein corecruitment. We here provide evidence that DNA-based protein micropatterning has clear advantages over simple protein patterning.

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Expansion Microscopy at the Nano-Bio Interface

M. Nakamoto[1], W. Zhang[1], and B. Cui[1]

[1]*Department of Chemistry, Stanford University, Stanford 94305, California, USA*

Advances in nanofabrication have opened the door for subcellular probing with nanostructures. This physical "nano-bio interface" formed between cells and nanomaterials is highly relevant for applications in medical implants, injectable hydrogels, and models of *in-vivo* extracellular matrix effects. Previous studies have shown that cells grown on 3D nanostructured surfaces exhibit a myriad of downstream effects including altered membrane trafficking, nuclear mechanotransduction, and cytoskeleton rearrangement.[1] These downstream effects depend on the geometry of the nanostructures and the resulting curvature the nanostructures impart on the cell membrane. Hence, high resolution viewing of biological phenomena at the nano-bio interface is highly desired. As nanostructures are generally fabricated to be 50-200 nm in diameter, optical microscopy with a resolution of ~200 nm is unable to resolve images to a desired extent. Here, we adapt and apply expansion microscopy (ExM) to selectively stain and view the nano-bio interface at a resolution of ~50 nm. Prior work has only used ExM to visualize cells grown on flat substrates.[2] We demonstrate that nanostructured surfaces very minimally affect isotropic ExM hydrogel encapsulation of the sample and subsequent expansion. Holes left behind by nanostructures in the gel expand with the same expansion factor as the overall gel. Using ExM to visualize the nano-bio interface at ~50 nm resolution opens the door for a plethora of cell-material interface studies.

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Non-cytotoxic, temperature-responsive and antibacterial POEGMA based nanocomposite coatings with silver nanoparticles.

S. Nastyshyn[1], Y. Stetsyshyn[2], A. Bernasik[3], B. Orzechowska[4], M. Brzychczy-Włoch[5], Y. Shybanova[2] and J. Raczkowska[1]

[1]Smoluchowski Institute of Physics, Jagiellonian University, Łojasiewicza 11, 30-348 Kraków, Poland

[2] Lviv Polytechnic National University, St. George's Square 2, 79013 Lviv, Ukraine

[3] Faculty of Physics and Applied Computer Science, Academic Centre for Materials and Nanotechnology, AGH University of Science and Technology, Al. Mickiewicza 30, 30-049 Kraków, Poland

[4]Institute of Nuclear Physics Polish Academy of Sciences, Radzikowskiego 152, 31-342 Kraków, Poland

[5] Chair of Microbiology, Department of Molecular Medical Microbiology, Faculty of Medicine, Jagiellonian University Medical College, Czysta 18, 31-121 Kraków, Poland

The polymer grafted brushes are macromolecular chains that are joined by one of their ends to the solid substrate. Nowadays they are widely used for commanding liquid crystals and proteins, for the fabrication of nanoparticles, and for other biological applications. High interest is paid to the so-called “smart” brush-coatings with thermo-switchable properties.

We fabricated poly(di(ethylene glycol)methyl ether methacrylate) (POEGMA) brush coatings with embedded silver nanoparticles having thermo-switchable antibacterial activity.

The antibacterial activity of POEGMA brush-coating with embedded silver nanoparticles was studied above and below the lower critical solution temperature (LCST) of POEGMA. We have found that below LCST all bacteria were alive whereas only dead bacteria were observed above the LCST.

We showed that silver nanoparticles embedded in POEGMA brush are not cytotoxic against human skin cells HaCaT and present weak, but detectable anticancer properties against melanoma WM35 cells.

To study the release of silver from the brush in the aqueous environment we performed the X-ray photoelectron spectroscopy (XPS) and established that the abundance of silver does not drop below 35% of the initial value even after 55 days of incubation.

To optimize the fabrication of thermo-switchable antibacterial POEGMA grafted brushes with embedded silver nanoparticles, we studied the thermal response of POEGMA grafted brushes of different thickness and different amount of embedded silver, measuring the temperature dependence of water contact angle. The experiments revealed a strong dependence of the thermal response of coatings on the amount of embedded silver and brush thickness.

Magnetic manipulation strategies towards cell signaling studies

A. Neusch[1], J.-S. Brand[1], M. Otten[1], I. Novoselova[1], M. Karg[1] and C. Monzel[1]

[1]*Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany*

Cellular signals rely on characteristic temporal and spatial distributions of signaling molecules, but hitherto it is unclear how their local presence and activity state affect the signaling outcome. To study these processes, innovative techniques enabling an active manipulation on subcellular scales are highly demanded. In recent years, Magnetogenetics emerged as an approach where novel magnetic nanoparticles (MNPs) and external magnetic fields are used to manipulate a single type of molecule to trigger various cellular processes [1].

Here, we compared different MNPs with regard to their prospective use as nanoactuators of cellular functions. On the one hand, a bioinspired semisynthetic nanoparticle - Magnetoferritin (MFt) - was chosen, which is based on the globular iron storage protein complex ferritin (Ft) that converts iron ions to a ferrihydrite core [1-2]. Using genetic modification, we produced Ft cages that contain only subunits with a ferroxidase center and synthesized a magnetic iron oxide core inside [2]. By using a well-defined protein cage as a scaffold, MFts are biocompatible, uniform in size and are equipped with a readily modifiable molecular shell. On the other hand, commercially available “nanoflower-shaped” iron-oxide MNPs (synomag, micromod, Rostock, Germany) were studied. Both particles were characterized via transmission electron microscopy, via electrokinetic ζ-potential measurements and in terms of their magnetic response. Different transfer methods into cells were carried out to probe the cell viability, the MNPs’ stability and their mobility under the influence of external magnetic fields. We then assessed how both MNPs complement each other with regard to their ability to manipulate the localization and activity state of signaling molecules and formulated perspectives towards nanomedical applications.

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Molecular Forces involved in Clathrin-Mediated Endocytosis of Nanoparticles and Viruses

Federica Pennarola[1], **Joel Christian[1]**, **Ulrich Schwarz[2]**, **Khalid Salaita[3]**, **E. Ada Cavalcanti-Adam[1]**

[1]Max Planck Institute for Medical Research, Department of Cellular Biophysics, Jahnstr. 29, 69120 Heidelberg, Germany.
[2]Institute for Theoretical Physics, Heidelberg University, Philosophenweg 19, 69120 Heidelberg, Germany.
[3]Department of Chemistry, Emory University, Atlanta, GA, USA.

The endocytosis of nanoscale particles, such as viruses, involves the tuning of chemical and physical signatures at the cellular interface. The entry mechanism depends not only on the mechanical properties but also on the surface chemistry of the particles and the local environment where host cells reside. It remains to be elucidated how both mechanical and chemical properties of viral particles are tuned to efficiently gain entry in host cells. Clathrin-Mediated Endocytosis (CME) is one of the best-characterized endocytic routes, in which vesicle formation is mostly driven by the self-assembly of the clathrin cage and the adhesion energy gained through receptor-ligand bonds, as can be described by an adapted Helfrich Hamiltonian[1,2]. Here we present a bottom-up approach, combining block copolymer micellar nanolithography, live-cell super-resolution microscopy, molecular tension probes and surface chemistry, to simultaneously control receptor clustering with nanometer precision while recording endocytic forces with pN force resolution at the single molecule level. To measure forces, we used DNA-based integrative tension sensors (ITS), covalently bound to gold-nanoparticles (AuNPs), and immobilized in a controlled manner on a sub-100 nm spatial resolution 2D ordered pattern[3]. The AuNPs have a diameter of 20-1000 nm, and are coated with proteins or virus-mimicking peptides. The critical diameter for inducing CME in a receptor independent manner is in the range of 100-300 nm[4], while small objects such as 20 nm Nps induced CME only when coated with molecules known to activate the pathway[4]. Our studies allow to observe, decouple and quantify the chemical (e.g. receptor-ligand binding, receptor clustering and protein recruitment) and mechanical (e.g. nanoscale-induced membrane deformations) contributions to the uptake process through force measurements in these different physio-chemical conditions, and deduce dynamical equations using our Hamiltonian approach.

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Nanoscopic organization of Wnt signalsomes for interrogation and manipulation of downstream signaling

Michael Philippi[1,2], Isabelle Watrinet[1], Marie Kappen[1], Sergej Korneev[1], Andreas Plückthun[3], Jacob Piehler[1,4*], Martin Steinhart[1,2,4*], Changjiang You[1,4*]

[1]Department of Biology/Chemistry, Osnabrück University, Osnabrück, Germany
[2] Institute for Chemistry of New Materials, Osnabrück University, Osnabrück, Germany
[3] Institute of Biochemistry, University of Zurich, Zurich, Switzerland
[4] Center of Cellular Nanoanalytics, Osnabrück University, Osnabrück, Germany

Spatiotemporal organization of signaling complexes at the plasma membrane plays an intricate role in regulating cell signaling. Unraveling the subtle interplay of molecular interaction of receptor subunits and effector proteins in the context of hierarchical micro-compartmentation in the plasma membrane remains a highly challenging task. For this purpose, we have here established spatial organization of signaling complexes in the plasma membrane of live cells based on surface functionalization by capillary nanostamping. Based on surface biofunctionalization with a bifunctional anti-GFP clamp, capturing of GFP-tagged protein into high-density nanodot arrays with a characteristic spot diameter of ~300 nm was achieved. This technique was utilized to explore the determinants of Wnt signalosome formation. Upon culturing cells on nanopatterned substrates, GFP-tagged Wnt co-receptor Lrp6 expressed at the surface of living cells was successfully assembled into nanodot arrays. Strikingly the co-receptor Frz as well as the cytosolic scaffold proteins Axin1 and Disheveled2 were spontaneously recruited into the nanodot array to form spatially defined signalosomes in the absence of ligand. Immunofluorescence staining confirmed ligand-independent Wnt/β-catenin signaling activated the nanodot arrays. The observation of synergistic enrichment of Wnt receptors and effectors on the nanodot arrays suggests that liquid phase separation of Axin1 and/or Dishevelled2 plays a key role in signalosome formation and downstream signaling.

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Mechanical Stimulation with Nanoscale Actuators Provides Scalable Spatiotemporal Control of Muscle Cell Biology

A. Ramey-Ward[1], H. Su[2], and K. Salaita[1,2]

[1]*Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology/Emory University, Atlanta, GA, USA*
[2] *Department of Chemistry, Emory University, Atlanta, GA, USA*

Cyclic application of force has been well studied as a mechanism to enchance myogenesis. Clinically, this occurs through exercise, and recent work *in vitro* has developed bioreactors that apply cyclic strain through fluid flow or substrate deformation. These techniques, however, apply strain to full tissues or cell monolayers, limiting the ability to study the role these forces play at the single-cell level. In this work, we used optomechanical actuators (OMAs), ~0.6 μm photothermally responsive nanoparticles made of a gold nanorod (100 × 20 nm) surrounded by a polymeric hydrogel shell modified with cell adhesion ligands. OMAs rapidly collpase when illuminated with near-infrared (NIR) light (785 nm), and we used this mechanical force to apply cyclic stimulation to C2C12 myoblasts with varying frequency, duration, and spatial patterning. Through these unique parameters of our mechanical assay, we found that myoblasts begin to respond to mechanical stimuli within 10 minutes through cytoskeletal changes, and show enhancement of nuclear YAP1 accumulation within 20 minutes, indicating mechanotransductive signaling within the cell. Repeated OMA mechanical stimulation for 5 days led to enhanced cellular alignment, fusion, and sarcomeric myosin expression in myotubes, morphological and biochemical hallmarks of myogensis. This OMA-mediated myogenesis was sensitive to the geometry of stimulation, demonstrating the unique parameter space of this assay compared to the axial stimulation of bioreactors. However, this nanoscale spatial resolution can pose a challenge in promoting myogenesis on the tissue scale. Therefore, in our recent work, we have covalently cross-linked OMAs into a biological hydrogel material, scaling up the mechanical response to stimluate many cells at once using the same photothermal actuation mechanism. This highly tunable responsivity across multiple length scales shows the versatility OMAs as a tool for manipulating cell biology *in vitro*, with potential for theraputic applications.

Investigation of nanoscale collagen films by SFA

Andreas Rohatschek[1#*], **Bruno Zappone**[2] **and Philipp J. Thurner**[1]

[1] Institute of Lightweight Design and Structural Biomechanics (ILSB), TU Wien, 1060 Vienna, Austria

[2] Consiglio Nazionale delle Ricerche – Istituto di Nanotecnologia (CNR-Nanotec), 87036 Rende (CS), Italy

[#]Recipient of a DOC Fellowship of the Austrian Academy of Sciences at the ILSB

Collagens are the most abundant and structurally the most important proteins of the human extracellular matrix. Taken this into account and considering the etymological origin of collagen (from greek "*Κόλλα*"- glue), the current lack of knowledge in literature about the adhesive properties of collagen molecules is surprising. Here, we present an approach to experimentally characterize tropocollagen-substrate and tropocollagen-tropocollagen interactions using the Surface Force Apparatus (SFA).

SFA measurements were conducted on type III collagen films with thickness smaller than 10 nm. Freshly cleaved sheets of muscovite mica were used as substrates in a crossed-cylinder single-contact geometry. Tropocollagen films were adsorbed on substrate surfaces either one-sided or two-sided from acid aqueous solution (pH 2). Measurements where performed in acetic acid (pH 3) or physiological-like buffer PBS (pH 7) at room temperature.

Fig.1 shows characteristic force-distance curves obtained during a cycle of surface approach-retraction. In acidic conditions, purely repulsive force and strong adhesion was detected in the two-sided setting. For the one-sided setting a decrease in adhesion but an increase in repulsion was observed indicating a transfer of molecules onto the opposite uncoated surface. In physiological-like conditions, the layers were swollen and the force was purely repulsive for the two-sided adsorbed setting. An interesting observation considering that under this condition self-assembly should be promoted. Maybe adjustment of additional environmental conditions (Temperature, time) has to be considered for future experiments to further investigate this behavior in more detail.

Within this study we could provide first data on tropocollagen-tropocollagen and tropocollagen-substrate interactions which are thus far in good agreement with other SFA studies on protein mediated adhesion [1].

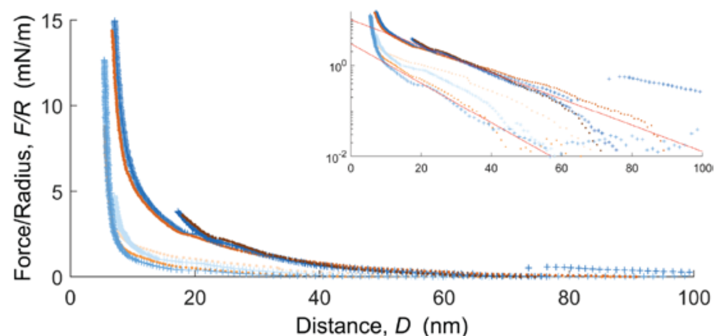

Figure 1. Force-distance curves at the same contact point, *Inset:* Semi-log representation of Force/Radius

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Conformation and Dynamics of Bone Morphogenetic Protein (BMP-2): Role of Extracellular Matrix (ECM) based Nanoparticles and Minerals

Harekrushna Sahoo

Department of Chemistry, National Institute of Technology-Rourkela, Rourkela-769008, Odisha (India)

Bone Morphogenetic Protein 2 (BMP-2), an osteoinductive and a bone extracellular matrix (ECM) protein, gets heavily affected by the alternation in the physical state of ECM components (such as minerals). Thus, the main objective is to unravel the impact of these components on BMP-2 conformation and dynamics. To elucidate the objective, hydroxyapatite nanoparticles (HAp NPs) which is the most abundant bone mineral and the other being a trace mineral *i.e.*, zinc oxide nanoparticles (ZnO NPs) are used as the potential nanoparticles for this study. On the other hand, different monovalent and divalent cationic salts of sulphates and phosphates are employed to investigate the impact. It is found that in the presence of HAp NPs and ZnO NPs, the interaction with the protein shows an upsurge. Comparatively, it is revealed that ZnO NPs are having more dominant secondary structure and thermal stabilizing effect to that of HAp NPs as evident from secondary structure analyses. Contrary to the impact of nanoparticles, different monovalent and divalent cationic salts of sulphates and phosphates also found to affect the conformation and stability of BMP – 2 as evident from the binding parameters. Thermal-dependent study revealed the major stability of BMP-2 upon interaction with phosphate salts; however, it did not vary significantly in case of sulphates in general.

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A FRET-based sensor for probing forces exerted by single T cell receptors on their ligands

Lukas Schrangl[1], Janett Göhring[1,2], Florian Kellner[2], René Platzer[2], Enrico Klotzsch[1,3,4], Hannes Stockinger[2], Johannes B. Huppa[2], Gerhard J. Schütz[1]

[1]Institute of Applied Physics, TU Wien, Vienna, Austria
[2]Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria
[3]Laboratory of Applied Mechanobiology, Department for Health Sciences and Technology, ETH Zürich, 8093 Zürich, Switzerland
[4]Institute of Biology, Experimental Biophysics/Mechanobiology, Humboldt Universität zu Berlin, 10115 Berlin, Germany

T cells employ T cell receptor (TCR) complexes to probe antigenic peptide presented on the surface of antigen-presenting cells (APCs). This interaction is of fundamental importance for the adaptive immune system as it constitutes the basis for the specific identification of harmful substances. Despite its importance, however, the mechanisms behind this recognition process have remained elusive. Prompted by indications suggesting that mechanical forces play a key role, we developed a FRET-based molecular force sensor to be inserted into the immunological synapse.
The sensor is composed of an anchor unit, a spring-like peptide derived from spider silk, and a TCR-binding ligand. The peptide, acting as a molecular spring, has donor and acceptor fluorophores attached near the opposing termini. Force-induced changes in the end-to-end distance can thus be monitored via the FRET efficiency.
As surrogate APCs, we functionalized supported lipid bilayers (SLBs) with force sensors featuring either antibody-derived single chain fragments (scF_v) or stimulatory pMHC as TCR-binding ligands. Forces arising from receptor–ligand interactions were recorded via a combined single-molecule FRET and tracking approach using TIRF microscopy.
Using strongly binding scF_v-functionalized sensors, we observed forces of 4–8 pN on gel-phase SLBs, where the sensors were virtually immobile. In experiments on liquid SLBs, allowing for free diffusion of sensors, only weak pulling events were found. Analysis of force magnitude with respect to the duration of the T cell–SLB interaction revealed an increase of forces after 10 minutes under high ligand density, activating conditions and a steady decrease of forces under conditions featuring a low ligand density emulating T cells scanning APC surfaces. Examination of single-molecule force traces yielded an approximately linear force increase of 1.5 pN/s for 2.5 s before being released in a single step.
When employing a stimulatory pMHC-functionalized sensor, forces around 2 pN were observed under activating conditions on gel-phase SLBs, while we did not detect any forces under scanning conditions.

Reconstituting PLC$_{\beta}$ Domain Function using Supported Lipid Bilayers

Kevin L. Scrudders[1], Kaushik Muralidharan[1], Angeline Lyon[1] and Shalini T. Low-Nam[1]

[1]*Department of Chemistry, Purdue University, West Lafayette, USA*

Phospholipases are lipolytic enzymes that convert phospholipids into fatty acids and lipophilic molecules. The generation of second messengers IP_3 and diacylglycerol by phospholipase C beta (PLCβ) hydrolysis of inner leaflet PIP_2 triggers calcium fluxes and protein kinase C activation. Given increased expression of PLCs in cardiomyocytes under pathological conditions and dysregulation in other diseases, the structure and function of these enzymes has been the subject of extensive study.[1] To better understand the role of the membrane in regulating PLC recruitment and activity we prepare reconstituted supported lipid bilayers and measure single-molecule PLCβ binding in a total internal reflection fluoroscence configuration. Flourescent protein-tagged PLCβ is captured from whole cell lysates to overcome the need to purify the protein and to measure contributions from normal cellular modifications or macromolecular complexes.[2] Molecular binding dwell times are measured as a function of membrane composition and PLCβ domain composition. Single enzymes are highly sensitive to substrate density and intramolecular regulation. These measurements create a springboard to measure the binding kinetics and activity of PLCε which is subject to more complex regulation and remains uncrystallized as a full-length construct.[3]

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Design of experiments to fabricate hydrogels by visible light photopolymerization using a 3D bioprinter

Valentina Serrano Cruz[1,2,#], Livia Neves Borgheti-Cardoso[1,#], Luciana Fontes de Oliveira[1], Nuria Torras Andres[1]; Elena Martinéz[1,2]

[1] *Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain*
[2] *University of Barcelona (UB), Barcelona, Spain*
[#] *Equal contribution*

Hydrogels have been investigated as a simple strategy to engineer 3D models because they provide friendly environments for cell growth and differentiation with a broad range of mechanical properties[1]. They can be formed by the photocrosslinking of polymers in the presence of a photoinitiator and light source. Compared to UV sensitive photoinitiators, visible light-sensitive photoinitiators are less cytotoxic and are highly soluble in water[2]. However, polymerization is a complex process, and find the proper combination of prepolymer solution composition and printing parameters can be time and money consuming, thus the use of Design of Experiments (DOE) is useful on optimization process and find variables that interfere more in the answers[3]. With this in context, this study aims to use experimental design to explore the process and try to find the best combination to fabricate photo-crosslinkable gelatine methacryloil (GelMA)—poly(ethylene glycol) diacrylate (PEGDA) hydrogels in the presence of a visible light source and the photoinitiator eosin Y, the co-initiator triethanolamine (TEA) and co-monomer N-vinylpyrrolidone (NVP). To define the best concentrations and printing parameters for the hydrogel, two factorial designs 2^3 and 2^4 were carried out. In both cases, the normal layer exposure time was the most significant factor (~78%) and good-shaped hydrogels were obtained when its value is shorter. Related to the prepolymer solution composition, better results were obtained when TEA concentration was lower (Fig.1). Thereby, the use of DOE was suitable to setup good parameters for fabricating the hydrogels. The viability of NIH-3T3 fibroblasts embedded into the optimized hydrogels was also evaluated 1 and 7 days after encapsulation by LIVE/DEAD assay, resulting in high viability values (>90%) (Fig.2).

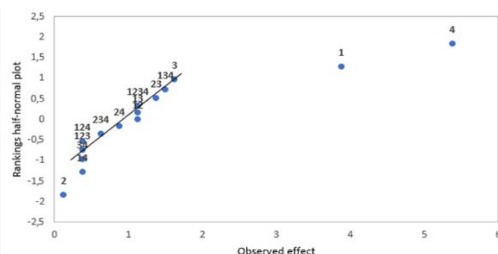

Figure 1. Half normal plot for the design 2^4. (1) Prepolymer solution; (2) Layer thickness (µm); (3) Bottom layer exposure time (sec); (4) Normal layer exposure time (sec).

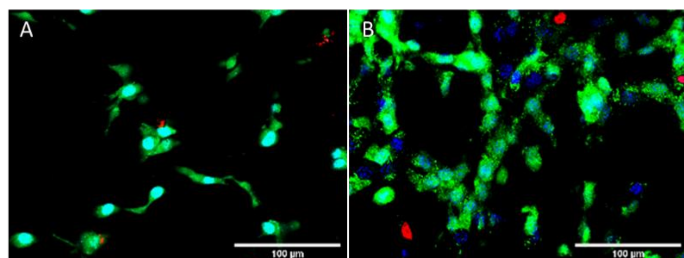

Figure 2. Live/Dead assay of NIH-3T3 cells embedded into the hydrogen (GelMA/PEGDA/EosinY/TEA/NVP) day 1 (A) and 7 (B) after bioprinting.

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Investigating the influence of biophysical properties of the environment on tumor cell dormancy

A. Shrivastava[1], **C. Chatterjee**[1], **and C. Lee-Thedieck**[1]

[1]Leibniz University Hannover, Institute of Cell Biology and Biophysics, Hannover, Germany

Disseminated tumor cells can hide in specialized distant niches and become “dormant”. These cells can remain inactive, which protects them from many chemotherapeutic treatments. Even long after the seemingly successful treatment of a primary tumor, these cells are able to reactivate and lead to a metastatic relapse at a secondary site. Dormant tumor cells are difficult to detect and pose an important challenge in the success of ongoing cancer therapies. At the same time targeting dormant cells before a metastatic relapse could be a potential strategy to fight cancer[1]. To do so, we certainly need to enhance our fundamental understanding of tumor cell dormancy. Previous works suggested the importance of the organ microenvironment in driving dormancy and the reactivation of dormant cells[2]. Also, it is evident from past researches that mechanical properties of the tumor microenvironment are known to play an important role in tumor progression and metastasis[3]. The aim of the presented project is to investigate the influence of biophysical properties of the environment on tumor cell dormancy. Previously, our group developed a polymer model system for mechanosensitivity studies with tunable mechanical properties which allows studying the influence of molecular extensibility on cell behavior[4]. With the help of this system, the potential influence of molecule extensibility on tumor cell dormancy will be investigated. The results obtained would expand our understanding of cancer cell dormancy and could be helpful in the development of new strategies to target cancer.

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Development of an Electroactive Platform for Detection of Virus Fusion to Host Membranes

Tiffany Tang[1], Achilleas Savva[2], Walther C. Traberg[2], Cheyan Xu[1], Quentin Thiburce[3], Han-Yuan Liu[1], Anna-Maria Pappa[2], Alberto Salleo[3], Roisin Owens[2], Susan Daniel[1]

[1]Robert F. Smith School of Chemical and Biomolecular Engineering, Cornell University, Olin Hall, Ithaca, New York 14853, United States
[2]Department of Chemical Engineering and Biotechnology, University of Cambridge, Philippa Fawcett Drive, CB30AS Cambridge, United Kingdom
[3]Department of Materials Science and Engineering, Stanford University, 496 Lomita Mall, Stanford, California 94305, United States

Every year, new virus outbreaks are reported and have potential to cause pandemics with devastating socio-economic impacts, with COVID-19 serving as a prime example. These outbreaks alluded to the importance of rapid viral sensing strategies to mitigating its impact and spread. PCR, one of the most common viral detection methods, is a specific and sensitive detection technique, but requires trained personnel and sophisticated machinery, slowing response time. An ideal detection method should integrate the specificity and sensitivity with the speed necessary to diagnosis patients rapidly.

Enveloped viruses (such as influenza virus, coronavirus) enter host cells by binding to a receptor and then fusing its viral membrane to the host cell membrane to insert its genome. We propose a novel sensing platform that leverages the virus's propensity to bind specifically and fuse to a host cell membrane and detect for changes to host cell membrane resulting from viral fusion. We can couple a biomimetic membrane on top of an electrically conductive polymer (PEDOT:PSS) to probe the electrical responses of the system once viral fusion has occurred. Using influenza virus as a proof-of-principle, we first show that we can form the biomimetic membrane incorporating the influenza viral receptor on PEDOT:PSS. We then visualize influenza virus fusion on PEDOT:PSS and demonstrate that the fusion kinetics are comparable to those on a non-conductive polymer. Lastly, using electrical impedance spectroscopy, we provide evidence that influenza viral fusion causes a detectable change in electrical response, demonstrating the possibility of exploiting viral membrane fusion for diagnostics.

DNA nanotechnology approaches to map and control the nano-organisation of membrane proteins

Ana I. Teixeira, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Sweden

Membrane proteins are the targets of more than 60% of all drugs currently in clinical use. Therefore, it is critically important to understand the biology of membrane proteins and the key biochemical and biophysical variables that regulate their function. Superresolution microscopy has revealed that most membrane proteins are not randomly distributed at the plasma membrane but instead localise to domains of nanoscale dimensions. Further, many receptors reorganize at the cell membrane upon ligand binding, suggesting that the spatial organization of membrane proteins has functional significance.

Our approach is to take advantage of the precision of DNA self-assembly to form nanostructures that enable detection or manipulation of protein spatial distributions at the cell membrane. To map protein nanoenvironments, we developed NanoDeep, a method that converts protein nanoscale spatial organization information into a DNA sequencing readout and provides unbiased ensemble information across cell populations with superresolution. To interrogate the functional roles of the spatial organization of membrane receptors and their nanoenvironment, we use DNA origami/ligand nanostructures, which we have previously shown to be uniquely suited to manipulate the spatial organisation of ligand/receptor interactions.

The overarching goal this work is to determine whether tailoring the nanoscale spatial organization of membrane proteins can lead to the development of new types of nanoassembled drugs.

Nanochip for personalized assessment of checkpoint immunotherapy

Esti Toledo[1,2], Guillaume Le Saux[1,2], Uzi Hadad[2], Angel Porgador[3], Mark Schvartzman[1,2]

[1] Department of Materials Engineering, [2]Ilse Katz Institute for Nanoscale Science & Technology, [3]The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel.

Immune checkpoint blockade has been extensively explored as a novel and promising antitumor treatment, these checkpoints help keep immune responses from being too strong and sometimes can keep T cells from killing cancer cells. When these checkpoints are blocked, T cells can kill cancer cells more efficiently. Yet this is largely challenged by low frequency of response, and the risk of developing autoimmune side effects. Whether and how the regulation of T-cells by the clustering of activating and inhibitory receptors is tumor dependent, and patient dependent is still to be investigated. Furthermore, does this regulation correlates with the patient sensitivity to PD-1 blockade by a commonly used PD-1 blocker Pembrolizumab (KEYTRUDA)? If it does, can it be exploited to predict patient responsivity to the checkpoint blockade?

To address these questions, we developed nanochip devices for the precise assessment of anti-tumor immunotherapy. These devices were designed with various nanoclusters of activating and inhibitory ligands which engage the receptors of T-cells. These nanochips were used as an artificial tumor cell, and help test T-cells from different patients on different nanochip arrays, with and without the PD-1 blocker drug Pembrolizumab, in order to study how the T-cells response to different patterns of the ligand clusters correlates with patient sensitivity to PD-1 blockade. This research has provided an important insight into the fundamental mechanisms of immune checkpoint blockade, and pave the way to the development of a novel nanochip technology for the personalization of this promising antitumor immunotherapy.

Substrate stiffness effects on neuronal cell culture *in vitro*

N. Wanders[1], M.F.J. Fransen[1], E.A.L. Raaijmakers[1], dr. S. Xie[2], and dr. R. Luttge[1]

[1]*Department of Mechanical Engineering, Microsystems Group, Eindhoven University of Technology, Eindhoven, The Netherlands*
[2]*MESA+ Institute for Nanotechnology, University of Twente, Enschede, Netherlands. Current address: Paul Scherrer Institute, Switzerland*

It is proposed that soft substrates, just like in native neural tissue, stimulate neuronal extension and viability *in vitro* [1]. Neuronal extension is a measure for neuronal differentiation. However, the discussion remains if neuronal extension equals network enhancement. Calcium imaging provides us an insight in the calcium-dependent activity of neurons and can therefore give an indication of neuronal network activity [2]. Human-derived neuroblastoma SH-SY5Y cells have been seeded on actuator chips. These chips are composed of three polydimethylsiloxane (PDMS) parts: the underneath channel layer, a membrane and a culture chamber layer. The thin PDMS membrane (10 µm) resulted in a softer culture substrate atop of the channels compared to the rest of the chip. Results have been obtained at 11, 18 and 25 days *in vitro* (DIV). Cell activity was quantified as spike rate over time via calcium-dependent fluorescence intensity with CALium IMaging Analyzer software (CALIMA) [3]. Cells on- and off-channel, soft and stiff substrate respectively, showed comparable spike rates over time at 11 DIV. After 18 DIV, cells on the softer substrate showed variation in spike rates, whereas cells on the stiffer substrate showed continuous activity over time. At 25 DIV, cells on the stiffer substrate continued to show similar activity as at 18 DIV, while spike rates of cells on the softer substrate diminished. Hippocampal neurons cultured on variating PDMS stiffnesses showed similar increased network activity on stiff PDMS [4]. In contrast, data from previous published work from our group showed that SH-SY5Y cells at 21 DIV spike less regular on the glass substrate vs flat PDMS; stiff and soft substrate respectively [5]. The chip allows us to alternate substrate stiffness without changing the intrinsic material properties in the same culture reservoir. In future experiments we want to explore such stiffness change to study the relation between maturation and network formation.

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Upscaling Modular tissue Engineering

G. Weisgrab[1 2], O. Guillaume[1 2] and A. Ovsianikov[1 2]

[1] Institute of Materials Science and Technology, 3D Printing and Biofabrication Group, TU Wien, Austria

[2] Austrian Cluster for Tissue Regeneration

The field of tissue engineering currently relies on two distinct approaches, either scaffold-based or scaffold-free cell culture. The first approach offers protected cell growth within a temporary structure at the expense of inhomogeneous cell seeding and an overall low initial cell number. The latter approach allows for initial higher cell densities and their homogeneous distribution at the cost of less controllable mechanical properties to favor rapid tissue formation. Recently, a third strategy was proposed combining both methods to provide tissue constructs with high cells density and biomimetic environment all the while shielding the bulk with a highly porous cage [1].

In this research, we show the fabrication of micrometer-sized porous cages using 3D printing via Two-Photon Polymerization and a biomaterial with a PCL backbone. We further show the formation of spheroids within these cages and the subsequent fusion of these building blocks to form larger tissues.

To generate a construct of a relevant size, a large amount of such single building blocks is required. Therefore, we demonstrate a microfluidic handling system to automate the separation, seeding and culture of single building blocks in an approach to reproducibly fabricate cell-laden scaffolds with a specific number of cells and thereby upscale modular tissue engineering.

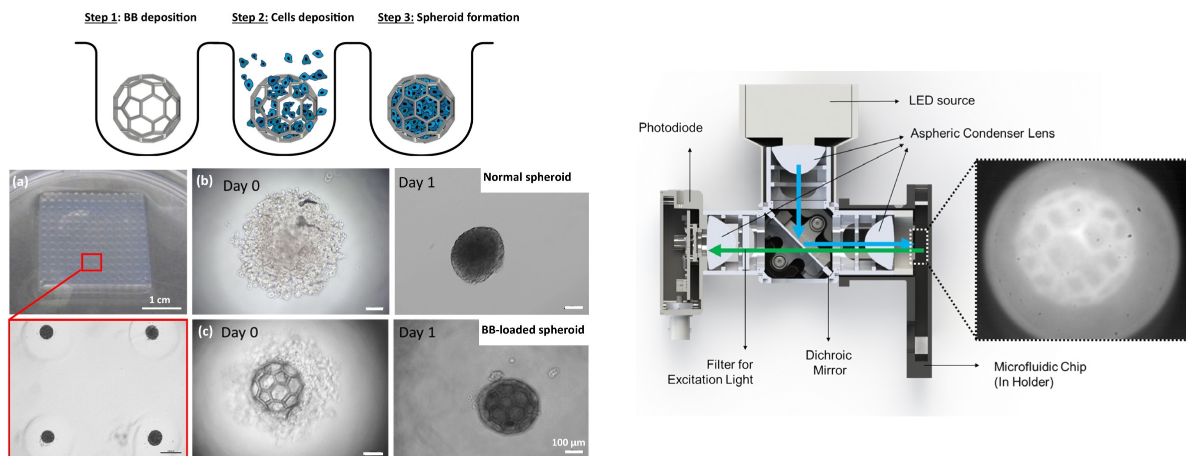

Fig1: Left: Cultivation Chambers for spheroid formation on microscaffold; Right: Automated setup for high-throughput sorting of 3D-printed microscaffolds

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Microchannel Cantilever Spotted Sensor Arrays for highly affinitive Indicator-Displacement Assays

C. Zhong,[1,2] C. Hu,[1] R. Kumar,[1,2] Vanessa Trouillet,[2,3] Frank Biedermann,[1] and Michael Hirtz[1,2]

[1]Institute of Nanotechnology (INT), Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany
[2]Karlsruhe Nano Micro Facility (KNMF), Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany
[3]Institute of Applied Materials (IAM), Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany

The patterned immobilization of chemosensors into microarrays has often boosted application in diagnostics and environmental sensing applications.[1] While this is a standard approach for biosensors, *e.g.* with antibodies, other proteins and DNA, arraying is not yet adopted widely for supramolecular chemosensors which are still predominantly used in solution systems.[2] We introduce the patterned immobilization of cucurbit[*n*]urils (CB*n*) into multiplexed microarrays and elucidate their prospects for advancement of surface-bound indicator-displacement assays (IDA) to detect small molecule analytes. The microarrays were generated by microchannel cantilever spotting (µCS) of functionalized CB*n* and subsequent self-assembly of corresponding indicator dyes from solution. Enhanced sensitivity of surface-bound microarrays was established in demonstrations with small bioactive metabolites (spermine, amantadine, and cadaverine) compared to bulk assays. Furthermore, integration of the CB*n*/indicator microarrays into microfluidic channels provides an efficient way for real-time monitoring of the sensing process, allows easier handling and reduces need in analyte volume. The concept was further extended to differential sensing of analytes on diplex or multiplex CB*n*/indicator microarrays, opening-up a route to multi-component sensing of small molecule analytes in complex liquids.

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