Ventures in time and space

Professor Dr Doris Heinrich, Head of Fraunhofer ATTRACT Research Group '3DNanoCell' in Würzburg, Germany, and Professor of Biophysics at Leiden University, The Netherlands, discusses her research on cellular cytoskeleton dynamics and applications in regenerative medicine

Could you describe the basis of your research? How are you working to achieve your objectives?

My research focuses on control of cellular functions for use in regenerative medicine. The aim is to develop smart implants and 3D scaffolds for tissue engineering. In standardised diagnostic assays, designed for the specific control of targeted cell functions, my research teams apply chemical, mechanical and/or electrical cues to probe and trigger cell functionality. To reach the nanoscale, we control these cues with the aid of multifunctional nano-tools.

An in-depth understanding of cytoskeleton regulation in living cells is required for the implementation of such methods. Whilst the genome is the blueprint of all life processes, the interaction of each individual cell with its environment determines gene expression, and thus cellular functionality. Molecular concentration gradients and mechanical interactions with the 3D scaffold of the extracellular matrix influence the behaviour of living cells, including processes such as migration, cell division, differentiation and apoptosis.

In vitro 3D scaffolds are applied as substrates to build tissue using living cells. These scaffolds are designed in combination with nanoscale surface structures to achieve optimal cell invasion and adhesion. This ensures the permanent survival of living cells and therefore tissue formation, further opening the way to next generation smart implants.

What makes the cytoskeletal dynamics of living cells under defined external stimuli of particular interest to you?

Applying defined external stimuli to living cells enables the investigation of cytoskeletal reorganisation and hence cell behaviour, which is the first step towards understanding cellular functionality. The next step would be the engineering of tools to control cell functions. We intend to trigger cells to act in a defined way. For example, initiating cell migration towards regions in the brain, where a stroke has destroyed tissue and impaired brain function, will repopulate this region and restore full brain functionality.

You lead the Fraunhofer ATTRACT Group '3DNanoCell' in the development of standardised diagnostic assays for the management and control of cell functions. Could you provide some insight into this work?

Almost every cell type is specialised to certain functions within the human body, so its cellular environment is unique. The mechanical, chemical and electrical properties of the extracellular matrix ensure that cells perform their proper functions. Diseases are often due to an imbalance in this system, which arise from malfunction or death of human cells. To examine the mechanistic origin of diseases, it is important to culture cells in their native environment, or to simulate it as closely as possible. Thus, we engineer artificial nanoscale 3D environments adapted to specific cell types, in order to perform standardised drug screening and diagnostics.

What are you currently working on?

We are working on nanoscale drug delivery systems, targeted to individual cell types. We have also developed biophysical tools to apply spatio-temporally controlled cues. These include magnetic tweezers, to control intracellular transport, and advanced microfluidic devices, to control cell migration and differentiation. Fields of implementation are tissue engineering and smart implants.

How have you overcome the challenges you have faced in your research?

The biggest challenge we face is the interdisciplinarity of this project, and the necessity to work in different established scientific cultures. Having worked in all of these disciplines previously, I can now function as a 'translator' when communicating with people from different research fields.

Could you outline the applications of your research, and how do you predict the field will evolve over the next few years?

Applications of my research include novel products for regenerative medicine and *in vitro* diagnostics. These comprise standardised 3D nanostructured assays for drug screening and diagnostics, smart active implants, tissue engineering, and new biophysical systems for the control of cell functions.

My research groups are continuously expanding and I predict that regenerative medicine will grow rapidly. The demographic changes in society and the resulting rising costs of healthcare make new solutions urgently necessary. New generations of active implants, cell-based diagnostics and cell carrier systems address attractive industrial markets of the future.

Guiding cells towards regeneration

Our insight into cellular behaviour is advanced by profound understanding of cytoskeleton dynamics and cellular response to external cues. Control of cell functions will find wide applications in novel diagnostic assays and regenerative medicine

CELL MOTILITY RELIES upon the cell cytoskeleton and is crucial to most vital processes in the human body, including immune response, axon guidance and wound healing. A key component of the cytoskeleton is actin, a protein which can be present as a free monomer or as part of a semi-flexible polymer. Both forms are essential for the motility and contraction of cells, enabling cell migration, division and changes in shape.

Professor Dr Doris Heinrich, head of two teams, the Fraunhofer ATTRACT Group '3DNanoCell' at the Fraunhofer Institute for Silicate Research (ISC) in Würzburg, Germany, and her research lab at Leiden University, The Netherlands, studies the rearrangement of actin networks as part of a wider frame of work, which aims to understand cell behaviour. The ultimate goal is to control cell functions, which provides a truly exciting prospect for the future of medical research and healthcare.

CELL MOTION AND THE ROLE OF ACTIN

It is easy to think of the cells within our bodies as stationary building blocks, but that is far from the truth. Living cells move and change shape to carry out critical processes. The very survival of many cell types in the body depends upon their perpetual active motions, which require structural changes in the cytoskeleton.

Actin is of great significance to cellular dynamics. It allows the structure and elasticity of the cell envelope to be modified within seconds and couples with microtubules to control cell stability. The most dramatic large-scale motions of cells take place during their crawling on surfaces, powered by actin polymerisation at the leading edge, generating protrusion forces, and retraction of the rear of the cell by actin-myosin fibres. Migrating cells proceed to explore the environment by quasi-random walks composed of zig-zag like paths and local reorientation motions.

INVESTIGATED RESEARCH FIELDS

On flat substrates, several cell types exhibit amoeboid migration, characterised by successive pseudopod (cell lobe) protrusions, which represent search modes. It consists of two alternating modes: a random probing mode and a directed running mode, both of which derive from actin polymerisation near the plasma membrane. This two-state migration is similar to motion patterns in large animals and the 'run and tumble' motion of prokaryotes, highlighting the universal nature of this remarkably efficient search strategy.

Chemotaxis describes the phenomenon in which cells migrate towards an external chemical stimulus. The external gradient is sensed by the cell and transformed into an intracellular gradient, which activates a signalling pathway culminating in actin polymerisation. Identifying proteins in these signalling events is crucial to understanding disease.

Professor Dr Heinrich's research focuses on cytoskeletal dynamics under defined external stimuli. Her research group at Leiden University controls cells by artificial chemotactic stimuli, to force them into predefined states. They also investigate cytoskeletal reorganisation in 3D topological environments, intracellular diffusion and directed material transport[1,2] as well as single molecule tracking. Nanotechnonology greatly facilitates their research, as Professor Dr Heinrich explains: "We use nanotechnology to construct nanoscale capsules as drug carriers, nanomagnets and nanoscale 3D cell environments". Meanwhile, her team members of the Fraunhofer ATTRACT Group '3DNanoCell' are developing standardised diagnostic assays to control cell function through novel designs of material chemistry and surface topography.

EXPERIMENTAL SETUPS

Various experimental systems can manipulate chemotactic gradients. This has been improved by microfluidic mixing devices, a system implemented by the Heinrich lab. This advanced setup has several innovative features: it allows the exposure of entire cell populations to homogenous gradients and control over rapidly alternating but uniform gradients. The microfluidic gradient generator modifies the shape and position of concentration gradients in a time-dependent manner, unlocking possibilities for studies of cell response as a function of switching frequency[3].

Professor Dr Heinrich and collaborators applied stimuli to cell ensembles whilst monitoring the signalling events in single cells by fluorescence. Alternating time sequences of concentration gradients were applied to quantify the intracellular responses of the motile cell archetype *D. discoideum*, sharing many features with animal or human cells and ideally suited to test the experimental limits of fast gradient switching . The combined observation of actin polymerisation and cell shape changes during chemotactic migration enabled insight into the control of cell locomotion by cytoskeleton reorganisation.

In addition, the researchers studied the influence of gradient steepness on cell polarisation during starvation. This approach enabled the differentiation of molecular mechanisms in time, which reflects development.

FASCINATING RESULTS

The studies coordinated by Professor Dr Heinrich have yielded many exciting results. They reveal the

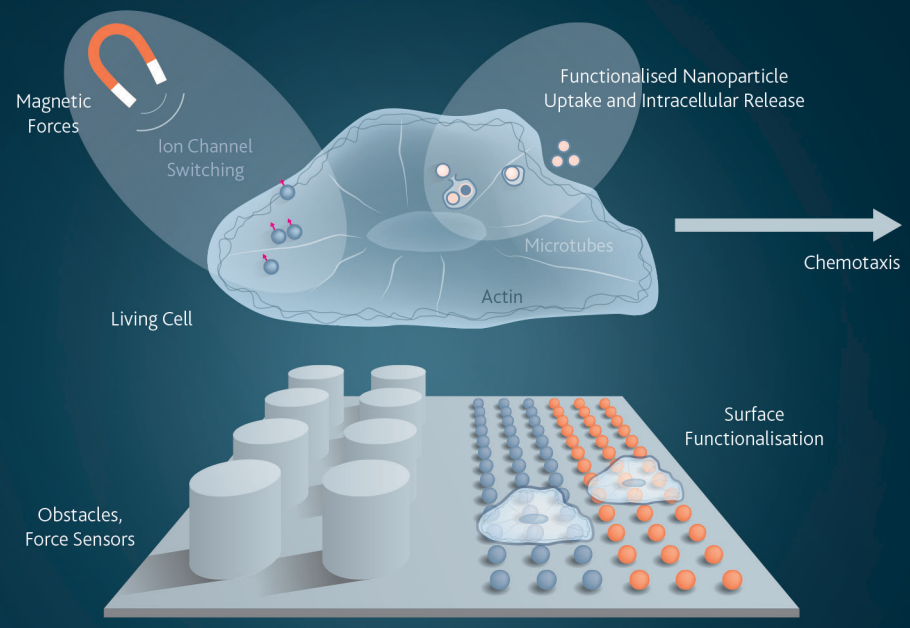

Figure 1: External cues, applied to living cells for use in regenerative medicine.

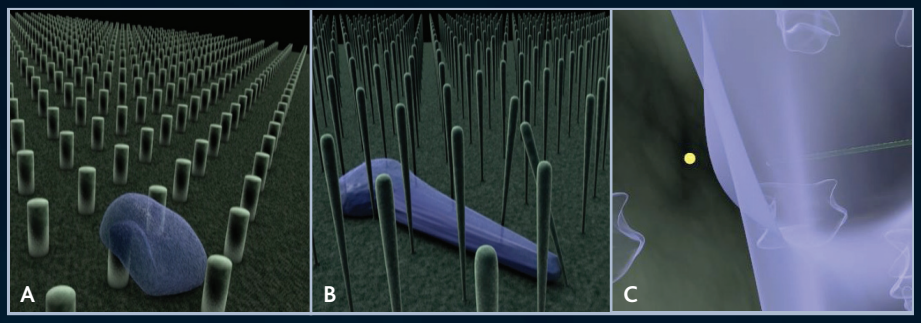

Figure 2: Animations of living cell behavior: **A)** Migrating cell (blue) in artificial 3D environment of pillar surface structures **B)** Living cell (blue) flexing surface topography **C)** View into a living cell during uptake of nanoscale drug carrier.

role of PI3K in cellular repolarisation and find two fundamentally different cell polarisation types.

Results[3] suggest that parallel molecular mechanisms are at play in *D. discoideum* chemotaxis signalling. The balance between the PI3K pathway (which enables fast reorientation of the cell) and the Pla2 pathway (which mediates persistent cell migration) appears to be strongly dependent upon gradient steepness and starvation time. In steep gradients, PI3K based formation of new pseudopods was primarily found. This decreased with starvation time as well as with a reduction of gradient steepness. In other words, there was a shift from PI3K based signalling to the Pla2 pathway. This could be because a rapid change in migration direction, as mediated by PI3K, is important for cells during early starvation, whereas it is more important for aggregating cells to persistently migrate towards the chemotactic source.

The team also quantified the influence of surface topography on migration. Microfabricated pillar arrays were used as a model of natural environments[4,5]. Results show that amoeboid migration modes are altered by the presence of 3D structures and describe two types of cell behaviour. Randomly moving cells remained in contact with pillars, whilst fast moving cells in a directed run phase were deflected by them. They also show that amoeboid cells migrate by maximising contact with available surfaces. Switching from randomly formed pseudopods to a stabilised, leading pseudopod is triggered by contact with surface structures. These alternating processes guide cells, leading to the development of a theory of 'contact-reinforced motility'.

This research provides valuable insights into cell motility, but there are enduring questions. The differences between cell migration on flat substrates and within a 3D topography remain a topic of continuing investigation. Further experiments into the redistribution of key cytoskeleton regulators are needed, and the role of microtubule-actin crosstalk in sensing and polarisation is yet to be clarified.

REMOTE MEDICINE

Professor Dr Heinrich believes these findings have huge potential. Her work suggests that in the not too distant future we may see medical approaches that are more targeted and less intervening.

Also, her research might have impact on other fields. The Heinrich lab has demonstrated that experiments using micro-structured surfaces in combination with quantitative analysis of cell motility reveal valuable information about cell-surface interactions and cell motility. This concept offers possibilities for cell sorting on a large scale, even for tissue parts, which would enable the study of collective cell motion.

The microfluidic gradient generator[3] enables the stimulation of single cells of entire developing organisms with specific molecular gradients. This technology is needed in a wide range of research areas and the flexibility of the generator allows for complex advanced assays in drug delivery and inflammatory response.

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INTELLIGENCE

SPATIOTEMPORAL CONTROL OF ACTIN NETWORKS BY EXTERNAL CHEMICAL AND TOPOLOGICAL CUES

OBJECTIVES

- Trigger cellular functions by external cues
- Control cellular functions by nano-tools
- Develop innovations in the fields of tissue engineering and smart implants

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CONTACT

Professor Dr Doris Heinrich

Leiden Institute of Physics, LION
Leiden University
Niels Bohrweg 2
2333 CA Leiden, The Netherlands

T +31 (0)71 527 4380
E heinrich@physics.leidenuniv.nl

www.physics.leidenuniv.nl/hl-home

and

Head of
Fraunhofer ATTRACT Group '3DNanoCell'
Fraunhofer Institute for Silicate Research (ISC)
Neunerplatz 2
97082 Würzburg, Germany

T +49 (0)931 3181862
E doris.heinrich@isc.fraunhofer.de

www.isc.fraunhofer.de

DR DORIS HEINRICH is Professor of Biophysics at Leiden University, The Netherlands, and Head of Fraunhofer ATTRACT Group '3DNanoCell' at the Fraunhofer ISC in Würzburg, Germany. She graduated with a Master's in Physics from the University of Texas at Austin, USA, and received her PhD in Semiconductor Physics from the Technische Universität München (TUM), Germany. Then, she performed her postdoctoral research in Los Angeles, Heidelberg and Munich with Professor Dr E Sackmann, investigating mechanical properties of living cells. Afterwards, she worked as a Business Consultant with McKinsey & Company on several projects in France, the Near-Middle East and the US. In 2007, she started her own research group at the Ludwig-Maximilians-University (LMU), Munich, Germany. Her research focus lies in the biophysics of living cell dynamics. Investigated systems include molecular motors *in vivo*, external stimuli leading to cell signalling, cell adhesion dynamics and lipid membrane organisation.

Fraunhofer

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