Frontiers in Material and Life Sciences

Book of Abstracts

CONFERENCE

FRONTIERS IN MATERIAL AND LIFE SCIENCES:

CREATING LIFE IN 3D

2-4 September, 2015

Brno, Czech Republic

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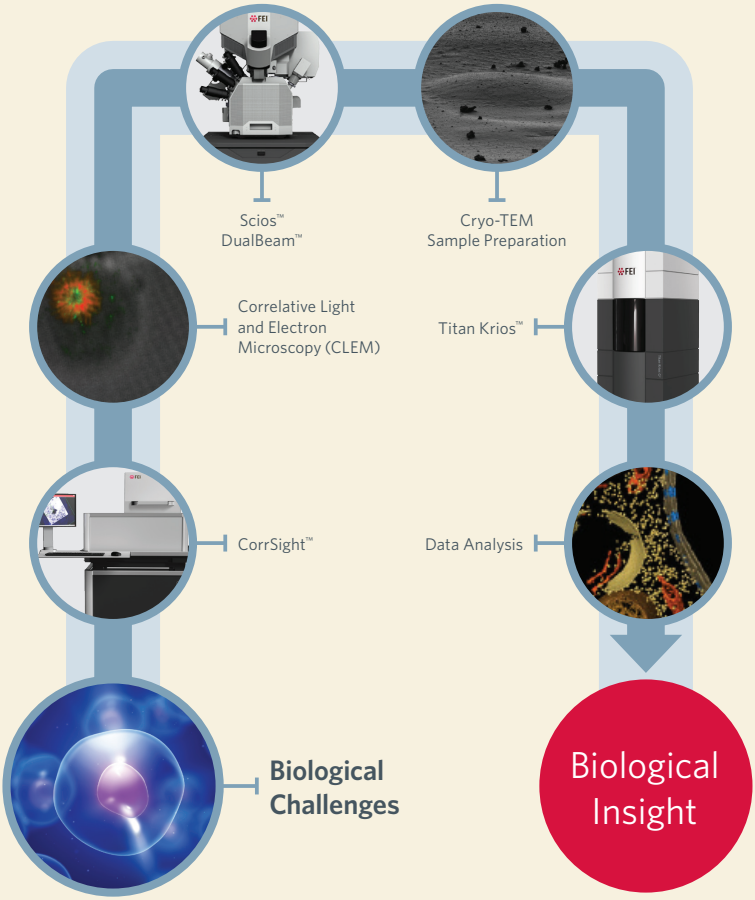

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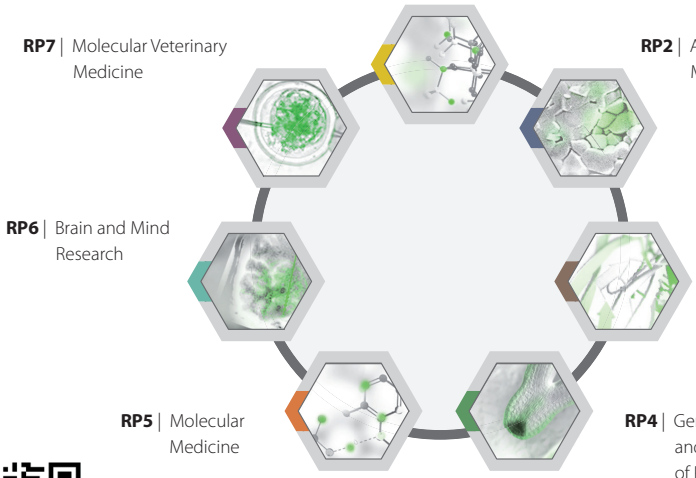
CEITEC is a scientific centre in the fields of the life sciences, advanced materials and technologies which aims to establish itself as a recognized centre for basic as well as applied research. CEITEC offers a state-of-the-art infrastructure and excellent conditions for the employment of outstanding researchers. It is a consortium of partners that include the most prominent universities and research institutes in Brno, Czech Republic: Masaryk University, Brno University of Technology, Mendel University in Brno, Institute of Physics of Materials of the Academy of Sciences of the Czech Republic, University of Veterinary and Pharmaceutical Sciences Brno and the Veterinary Research Institute. CEITEC works closely with the Region of South Moravia and the City of Brno to help increase local innovative capacity.

BASIC OVERVIEW

6 partners | 7 research programmes
61 research groups | 557 researchers (2015)
25 000 m^2 of new laboratories
10 core facilities

RESEARCH PROGRAMMES



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St. Anne's University Hospital Brno (in Czech: Fakultní nemocnice u sv. Anny v Brně - FNUSA) combines clinical practice, clinical research and education and its specialized departments serve as training and educational facilities for the Faculty of Medicine at Masaryk University and other faculties and training centers.

As the only university hospital in the Czech Republic, FNUSA has received funding from the European Structural Funds to create a European center of excellence – thanks to this grant, in 2012 FNUSA founded the International Clinical Research Center (ICRC), which is now its integral part.

International Clinical Research Center

The International Clinical Research Center of St. Anne's University Hospital Brno (FNUSA-ICRC) is a new generation scientific research center focused on effective prevention, diagnosis and personalized treatment of cardiovascular and neurological diseases – because these are among the most common causes of death in modern society.

The center is built on the foundation of many years of successful cooperation with the Mayo Clinic (USA) and many other foreign (mainly European) and also Czech partners.

FNUSA-ICRC employs over 350 research professionals who operate in 17 international scientific teams.

Project ICRC-ERA-HUMANBRIDGE

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ICRC–ERA–HUMAN BRIDGE

Conference "Frontiers in Materials and Life Sciences: Creating Life in 3D" took place also thanks to support of the ICRC-ERA-HumanBridge project, which has provided the conference with the necessary financial and organizational support. This project is focused on overall development of FNUSA-ICRC with aim to strengthen its position among other research centers in the European research area.

Project ICRC-ERA-HumanBridge has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 316345

WELCOME ADDRESS

Dear Participants of the Frontiers in Material and Life Sciences 2015,

It is a great pleasure to welcome you to Creating Life in 3D in Brno, Czech Republic. CEITEC is becoming more internationally recognized, as such, this conference will bring more than 25 international scientists as speakers to Brno. This meeting is designed to bring experts and students together from different disciplines to stimulate the formation of new collaborations. Also, this conference will provide a platform to showcase science from the Brno academic community.

The major goal of this year's conference is to address important scientific questions as we move from 2D models of living systems, to a more representative rendition of life in a 3D form. The transition to three dimensional living systems, by virtue of altering the architecture and composition of cells, displays different signalling and gene expression patterns, for example. A further level of complexity, three dimensionality presents, is to test and characterize materials which constitute the physical scaffolds of 3D systems. Flexibility, composition, and shape of these physical/chemical structures must enable living systems to grow, differentiate, and evolve, thus presenting temporal and spatial challenges. Moreover, the understanding of the chemistry at the interface between physical scaffolds and living cells will be examined. Finally, modelling such systems as well as their experimental reconstitution demand novel scientific approaches, which are being complemented at the level of innovative instruments, offering capabilities to image and characterize 3D experimental designs. We look forward to having scientists, students, and company stakeholders join us in this exciting field within the Material and Life Sciences theme. The conference will cover a breath of subjects including: stem cells, chemistry and physics of biomaterials, advanced instrumentation, cell-biomaterial interface, and 3D modelling.

As a social highlight, we will have a gala dinner in the spectacular Mendel Abbey, where Gregor Mendel made his ground-breaking discoveries to formulate the Principles of Inheritance. We hope this landmark setting will both inspire creative ideas and stimulate future interdisciplinary collaborations with new colleagues. This will also give us the opportunity to celebrate the winners of the poster competition and to taste wines from the South Moravian region.

We wish you a wonderful stay during the 2015 Frontiers in Material and Life Sciences Conference, **Creating Life in 3D**.

Markus Dettenhofer
Executive Director, CEITEC

WELCOME ADDRESS

Dear participants and readers,

Welcome to Frontiers in Material and Life Sciences: Creating Life in 3D. This event is the first conference organised jointly by CEITEC and FNUSA-ICRC and we are happy that is has drawn a number of renowned scientists from well-established research institutions as well as a number of student of great promise to Brno. We strongly believe that collaboration in research and education is the key to breakthrough discoveries and innovations, which will improve lives of millions of people worldwide.

Strong international collaboration is one of key features of the International Clinical Research Centre of St. Anne´s University Hospital, which is a relatively new research institution being built, since 2011, here in Brno with the financial support from the European Union and the government of the Czech Republic. Our researchers collaborate with counterparts from more than 100 universities, hospitals and research centres around the world. Most of our partners are located in Europe and North America, but we also collaborate with partners in Argentina, Australia and South Korea.

We have also managed to attract more than 50 researchers to Brno: currently we have employees from Argentina, Armenia, Austria, Germany, Hungary, India, Italy, Poland, Portugal, Russia, Slovenia, Slovakia, United Kingdom and the USA. They are all happy to work here in Brno. For all of us at FNUSA-ICRC, and likewise at CEITEC, this is an opportunity to build centres which are not merely new – both centres are also new-generation institutions which build their activities on multidisciplinary international collaboration and flexible research teams.

It not a coincidence that CEITEC and FNUSA-ICRC are being built just now here in the Czech Republic. The territory, which is now Czech Republic, gave to the world a number of discoveries and innovations, which significantly enhanced medicine and contributed to science in their own right. Mendel was already mentioned as the father of genetics. Purkyně was one of the founding fathers of cellular biology: his work helped formulate theory of cell and he discovered "Purkinje fibres" in the heart, which transmit nerve impulses and "Purkinje cells" in the cerebellum, which play a key role in motor coordination. Jansky discovered that human blood could be classified into four distinct groups and not three as previously thought. Czerny was the first physician to perform plastic surgery on female breast. Heyrovsky, the Nobel Prize winner, devised the polarographic technique, which has countless applications in chemical analysis, for example in toxicology. Wichterle manufactured first soft contact lenses and thus improved eyesight of millions of people. Holý discovered a compound, which was crucial for the development of important antiretroviral drugs used in the treatment of HIV and hepatitis B. And last, but not least, researchers of our hospital developed artificial heart valve only 3 years after first valve implant in the USA and independently of them.

Let me wish you enjoyable reading of proceeding from this conference. May this event inspire you – the world waits for your discoveries and innovations.

Gorazd B. Stokin
Chair of FNUSA-ICRC

POSTER SESSION

All participants of the **CEITEC / ICRC Conference** are invited to submit an abstract and poster which will be displayed during the whole conference. This is a unique opportunity to present your data to the international speakers, colleagues and other participants.
All posters must be based on relevant 5 topics to the conference.

Topics and sub-topics for poster session:

1) Stem cells: Genetics / Signalling

Cell differentiation, Cell reengineering, Intercellular signaling, Cell-to-cell communication, Genetics and epigenetics, Cell polarity

2) Chemistry and Physics of Biomaterials

Scaffolds and specific applications, Biomaterials fabrication, Tissue Engineering, Building blocks, Functional hydrogels, Nanoparticles and nanofibers

3) Advanced Instrumentation

3D/ 5D imaging of biological systems, Image data analysis and software, Stereoscopy, Correlative microscopy / High-Throughput EM

4) Cell-Biomaterial Interface

Surfaces andinterface, Simulation of life and material dynamics

5) 3D Modelling

Model systems for studying 3-dimensional life, Organ-on-a-chip, Computer modeling of 3-dimension complexity, In vitro organogenesis

Selection Criteria

Participants will be selected on the basis of the abstract submission. All selected participants will give their presentation in the form of posters. In addition five of abstracts will be selected for flash poster presentation.

Poster guidelines:

- Please bring one printed copy of your poster A0, portrait format.
- Posters should have a border of a few centimetres to allow the frame.

The participant is responsible for making sure that the poster display fits on the display board, and is completely responsible for attaching the individual elements to the display board according to our instructions.

PROGRAMME OF THE POSTER SESSION

Wednesday, September 2 18:30 - 20:00

Posters with odd numbers will be presented in this section

Thursday, September 3 17:20 - 19:40

Posters with even numbers will be presented in this section

PROGRAMME

Wednesday, September 2

07:30	Registration
	Welcome speeches
09:00	**Petr Vokřál, Petr Štěpánek** *(Czech Republic)*
09:10	**Gorazd Stokin** *(Czech Republic)*
09:20	**Markus Dettenhofer** *(Czech Republic)*
	SESSION 1.1 - Biology of stem cells, *Chair:* Aleš Hampl *(Czech Republic)*
09:30	**Igor Adameyko** *(Sweden)* - Novel insights into development of a face
10:00	**Ben Scheres** *(The Netherlands)* - Transcription factor gradients and plant stem cell progression
10:30	Coffee break
10:50	**Didier Montarrass** *(France)* - Skeletal muscle stem cells and regenerative myogenesis
11:20	**David Sassoon** *(France)* - Cell stress, stem cells and whole body metabolism
11:50	Lunch
	SESSION 1.2 - Biomaterials, *Chair: Lucy Vojtova (Czech Republic)*
13:20	**Alfred Crosby** *(USA)* - Polymer-Nanoparticle Mesostructures and Their Mechanics
13:50	**Simone Sprio** *(Italy)* - New bioceramics and hybrid composites in regenerative medicine

Wednesday, September 2

14:20	**Wolfdietrich Meyer** *(Germany)* - Polymeric Photocurables for artifical biocompatiblel blood-vessels structures
14:40	**Krzysztof Pielichowski** *(Poland)* - Organic-inorganic hybrid materials for biomedical applications
15:00	Coffee break
	SESSION 1.3 - Cell-Biomaterial Interface, *Chair: Markus Dettenhofer (Czech Republic)*
15:20	**Massimiliano Caiazzo** *(Switzerland)* - Defined 3D microenvironments boost induction of pluripotent stem cells
15:50	**Carsten Werner** *(Germany)* - Biofunctional Polymer Matrices for Stem Cell Bioengineering
16:20	**Joao Mano** *(Portugal)* **-** Hierarchical open and closed polymeric devices for regenerative medicine
16:50	Coffee break
	SESSION 1.4 - Computational Modeling (chairman: Josef Jančář), *Chair: Josef Jančář (Czech Republic)*
17:10	**Deok-Soo Kim** *(South Korea)* - Molecular Geometry and Its Operating System: A New Computational Paradigm for Understanding the Structure of Atomic Arrangements
17:40	**Andrey Milchev** *(Bulgaria)* - How computer simulations help understand the biomedical world
18:00	**Robert Vácha** *(Czech Republic)* - Passive endocytosis mediated by ligand-receptor interactions
18:20	*Selected poster presentation 1*
18:30	**Poster Session 1**

Thursday, September 3

	SESSION 2.1 - 3D instrumentation, *Chair: Martin Anger (Czech Republic)*
09:00	**Jason Swedlow** *(UK)* - Signalling and Mechanics in the Human Mitotic Spindle
09:40	**Pavel Hozák** *(Czech Republic)* - Novel functions of phosphoinosites in regulation of chromatin functions
10:10	**Roger Albert Wepf** *(Switzerland)* - Bridging Microscopes: 3D correlative light & scanning electron microscopy of complex biological structures
10:40	Coffee break
11:00	**Wim Voorhout** *(The Netherlands)* - 3D Imaging at the Nanoscale: unveiling the wonders of nature at the molecular level
11:15	**Daniel Smeets** *(Germany)* - Leica TCS SP8 STED 3X – Principle and applications of the next dimension in super-resolution microscopy
11:30	**Jan Hejátko** *(Czech Republic)* - Inspecting the role of hormonal crosstalk in the root gravitropic response via in vivo imaging at the sub-cellular resolution
11:55	**Martin Mistrik** *(Czech Republic)* - Application of advanced microscopic techniques in molecular biology
12:20	*Selected poster presentation 2*
12:30	Lunch
	SESSION 2.2 - Driving Stem Cells Behavior I, *Chair: Igor Adameyko (Sweden)*
13:40	**Stephen Dalton** *(USA)* - New models for tissue repair and engineering using human pluripotent stem cells

Thursday, September 3

14:10	**Heike Walles (Dr. Marco Metzger)** *(Germany)* - Engineering of 3D-Tissues
14:40	**Eran Meshorer** *(Israel)* - Endogenously labeled fluorescent protein libraries in embryonic stem cells
15:20	*Selected poster presentation 3*
15:30	Coffee break
	SESSION 2.3 - Biomaterials 2, *Chair: Krzysztof Pielichowski (Poland)*
15:50	**Alina Sionkowska** *(Poland)* - 3D materials based on biopolymers
16:20	**Werner E. G. Müller** *(Germany)* - Biomaterials and 3D-Printing: Potentials in Medical Applications
16:50	**Josef Jančář** *(Czech Republic)* - The effect of hydroxyapatite nanoparticles on the morphogenesis and properties of collagen I
17:10	*Selected poster presentation 4*
17:20	Coffee break
17:40	**Poster Session 2**
19:00	Gala dinner in Abbey (Mendel Square)

Friday, September 4

	SESSION 3.1 - Cell Biomaterial Interface, *Chair: Werner E. G. Müller (Germany)*
09:00	**Fergal O'Brien** *(Ireland)* - Advanced biomaterials & scaffold–based systems for the delivery of therapeutics: new frontiers in regenerating organs & tissues
09:40	**Prasad Shastri** *(Germany)* - Mechanobiology in the Vascular Development and Central Nervous System
10:20	*Selected poster presentation 5*
10:30	Coffee break
	SESSION 3.2 - Driving Stem Cells Behavior II, *Chair: Karel Souček (Czech Republic)*
10:50	**Andrew Ewald** *(USA)* - 3D models of breast cancer invasion and metastasis
11:20	**Oleg Lunov** *(Czech Republic)* - Regulation of stem cells machinery by high-gradient magnetic fields
11:35	**Irena Koutná** *(Czech Republic)* - Biodistribution of non-coated γ-Fe_2O_3 nanoparticles in stem cells
	SESSION 3.3 - 3D imaging, *Chair: Jozef Kaiser (Czech Republic)*
11:50	**Giulliana Tromba** *(Italy)* - Biomedical Imaging with Synchroton Radiation: the experience at the SYRMEP beamline of Elettra
12:10	**Brian Metscher** *(Austria)* - MicroCT Analysis of Embryos and Soft Tissues
12:30	**CLOSING WORDS,** *Markus Dettenhofer, Gorazd Stokin*
12:40	Lunch

ABSTRACTS OF SPEAKERS

Session 1: Stem cells: Genetics / Signalling

NOVEL INSIGHTS INTO DEVELOPMENT OF A FACE

Igor Adameyko
Karolinska Institutet, Stockholm, Sweden

Facial region represents highly integrated and still enigmatic compartment that demonstrates high degree of coordination between different cellular sources and differentiating structures during embryonic development. Building up the skeletal elements, especially in the head, has been extensively studied in the past. However, no consensus exists today about how embryos control precise and complex geometries of their cartilages, bones and joints. Our hypothesis implies that controlled allocations of the organized polarized cellular micro-domains provide precise sculpting and scaling up various cartilaginous structures in the body. We clearly see similar logic in activity of dental mesenchymal stem cells and cell fate choice in populations of pulp cells and odontoblast during teeth development and continuous growth. Relatively small geometrically simple or complex clonal envelopes serve as tissue units that can be increased in numbers or in size to scale up the structure without shape loss or drastic distortion. At the moment we succeeded with mathematical model simulating shape-related aspects of morphogenesis in 3D. In this model we can place progenitor cells anywhere in the virtual tissue, we can introduce migration, change cell division speed and allocation of daughter cells, use gradients of attractants and orienteers etc. The best feature of the model includes clonal analysis in 3D + time with mathematical assessment of order and geometry in the whole resulting tissue that is comparable to the experimental natural tissue dynamics. Understanding tissue dynamics during development and regeneration is a key for much future advancement. For example, hundreds of human mutations leading to congenital abnormalities have been identified. However, in most cases we are missing the understanding of what is happening between the gene and the phenotype, i.e. how the collective cellular behavior is translated into morphofunctional outcome. We are filling fill this conceptual niche through understanding cellular behavior and mechanisms in shape-making. In general, we utilize multiple transgenic animals allowing us to dissect the role of different signaling pathways in shape-making of skeletal elements. To experimentally address our hypothesis we also use advanced genetics tracing with multicolor reporters, single cell transcriptomics, mathematical modelling and 3D-imaging of the entire developing skeletal structures in the context of the whole body using micro-computed tomography (CT). At the moment we develop and upgrade techniques for high contrast micro-CT imaging that allows us segmenting cartilage and mesenchymal condensations with 50 micrometer resolution. In a pilot experimentwe developed a novel approach of contrasting embryos with salts of tungsten acid that provides significantly improved contrast. We also do microsurgery and grafting, live imaging of zebrafish as well as ex vivo organoid cultures with microfluidics-based artificial gradients to understand the logic of the fate induction and deposition of cartilaginous elements in space.

Session 1: Stem cells: Genetics / Signalling

MULTIPOTENT MESOTHELIUM PROGENITORS DERIVED FROM HUMAN PLURIPOTENT CELLS FUNCTION IN DEVELOPMENT AND TISSUE REPAIR

Miranda Hayworth[1,2], David M. Reynolds[1,2], Ian White[1,2], Michael Kulik[1,2], Laura Menendez[1,2], Thomas Colunga[1,2], Tatiana A. Yatkievych[3], Parker B. Antin[3,4], Kit Nazor[5], Jeanne Loring[5], and Stephen Dalton[1,2*]

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[2] Department of Biochemistry and Molecular, University of Georgia,
[3] Departments of Cell Biology and Anatomy and
[4] Department of Molecular and Cellular Biology, University of Arizona, Medical Research Building, 1656 E. Mabel Street, Tucson, AZ 85724, [5] Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037.
Email address of submitting author: sdalton@uga.edu

The outside lining of coelomic organs are composed of surface mesothelium that contributes to their underlying vascular network in addition to growth and function of the underlying tissue. In this report, we describe the efficient differentiation of human pluripotent cells (hPSCs) into mesothelial progenitor cells (MPCs). These cells resemble embryonic mesothelium at the molecular level and integrate into mesothelial layers of the heart, liver, gut and lung following transplantion into chick embryos. The epigenetic signature of MPCs predicts them to have vasculogenic and myofibroblast differentiation potential. Multipotency of MPCs was confirmed by clonogenic assays that demonstrate smooth muscle, endothelial and fibroblasts differentiation potential. MPCs transplanted to mechanically-damaged neonatal mouse heart migrate into damaged tissue along with endogenous epicardium-derived cells, as judged by Wt1-Cre lineage tracing, and assemble into coronary vessels in the repair zone. These findings raise the possibility that MPCs have potential utility for tissue repair of coelomic organs.

Session 1: Stem cells: Genetics / Signalling

Biodistribution of non-coated γ-Fe_2O_3 nanoparticles in stem cells

Irena Koutná[1*], Barbara Šalingová[1], Petr Synek[2], Lenka Zajíčková[2]
[1] *FI MU, CBIA, Brno, Czech Republic*
[2] *CEITEC, Plasma Technologies, Czech Republic*
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Superparamagnetic iron oxide nanoparticles have become a useful tool in biomedicine. Their applications include cell labelling for magnetic resonance imaging, drug and gene delivery and cell targeting. Targeting cells to a specific location is a first step in development of regenerative cellular therapy. To provide optimal dispersion, increase nanoparticle intake and minimize toxic effects Fe_2O_3 nanoparticles are often coated with different types of organic materials including citrate, dextran, albumin, starch polyethylene glycol or dimercaptosuccinic acid. However, magnetism of the nanoparticles decreases with diameter of the coating. Recently, the γ-Fe_2O_3 nanoparticles were synthesized in gas phase by microwave plasma torch without any surfactants and additives. Therefore, this technology can open new perspectives for bioapplications. We studied interaction of different cell cultures including pluripotent cells with microwave-torch γ-Fe_2O_3 nanoparticles. Our data indicate that these nanoparticles have minimal toxic effects and can be used in regenerative medicine.

Session 1: Stem cells: Genetics / Signalling

Regulation of stem cells machinery by high-gradient magnetic fields

Oleg Lunov[1*], Vitalii Zablotskii[1], Eva Syková[2], Šárka Kubinová[1,2] and Alexandr Dejneka[1]
[1] *Institute of Physics AS CR, Prague 8, Czech Republic*
[2] *Institute of Experimental Medicine AS CR, Prague 4, Czech Republic*
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Nowadays, the focus in medicine on molecular genetics has resulted in a disregard for the physical basis of treatment even though many diseases originate from changes in cellular mechanics.1,2 Perturbations of the cellular nanomechanics promote pathologies, including cardiovascular disease and cancer. Furthermore, whilst the biological and therapeutic effects of magnetic fields are a well-established fact, to date the underlying mechanisms remain obscure. Here, we show that oscillating HGMF and mechanical vibration affect adipogenic differentiation of mesenchymal stem cells (MSCs) by the transmission of mechanical stress to the cell cytoskeleton, resulting in F-actin remodelling and subsequent down-regulation of adipogenic genes.3 Our findings propose an insight into the regulation of cellular nanomechanics, and provide a basis for better controlled down-regulation of stem cell adipogenesis by HGMF, which may facilitate the development of challenging therapeutic strategies suitable for the remote control of biological systems.

Grant support: MEYS under NPU I: LO1309.

References
[1] Zablotskii, V., et al. PLoS One 8, e70416 (2013).
[2] Zablotskii, V., et al. Biomaterials 35, 3164-3171 (2014).
[3] Zablotskii, V., et al. Appl. Phys. Lett. 105, 103702 (2014).

Session 1: Stem cells: Genetics / Signalling

Endogenously labeled fluorescent protein libraries in embryonic stem cells

Eran Meshorer[1,*]
[1] *The Department of Genetics, The Institute of Life Sciences, and the Edmond and Lily Safra Center for brain Sciences (ELSC), The Hebrew University of Jerusalem, Jerusalem, Israel 91904; *meshorer@huji.ac.il*

Embryonic stem cells (ESCs), with their dual capacity to self-renew and differentiate, are commonly used to study differentiation, epigenetic regulation, lineage choices and more. We generated a library of over 200 clones of mouse ESCs using a gene-tagging approach, with each clone expressing a fluorescent tagged protein (YFP or Cherry) under the control of its own endogenous promoter. I will demonstrate the power of this library to track proteins in living cells; identify heterogeneously expressing proteins; measure the dynamics of endogenously labeled proteins; screen for proteins that are recruited to sites of DNA damage; screen for potential drugs, pull-down tagged fluorescent fusion proteins using anti-Cherry antibodies and test for interaction partners; and screen, using live imaging of differentiating clones, for novel pluripotency-related factors. Using the latter approach, we identified SET nuclear oncogene (SET), a multifunctional linker histone chaperone, which remarkably, shifts from the SETα to the SETβ isoform by alternative promoter usage during early ESC differentiation. Using knockdown and CRISPR/Cas9-mediated knockout approaches, we further show that SET is essential for active proliferation and differentiation of ESCs, and that SETα helps maintain a hyperdynamic chromatin state in ESCs while SETβ is essential during early neuronal differentiation. This work provides the first endogenously labeled fluorescent tag library in ESCs, and identifies a novel chromatin regulator of proliferation and differentiation in ESCs.

Session 1: Stem cells: Genetics / Signalling

Skeletal muscle stem cells and regenerative myogenesis

Didier Montarras
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The formation of skeletal muscle depends on myogenic regulatory factors of the MyoD family that are essential for entry into the myogenic programme and subsequent differentiation of muscle fibres. Attention has also focussed on upstream regulators of muscle progenitor cells, prior to activation of the myogenic determination genes *Myf5* and *MyoD*. In this context, Pax3 and Pax7 play an important role. In the embryo, Pax3 marks muscle stem cells that give rise to trunk and limb muscles. Pax7 is subsequently co-expressed with Pax3 in this cell population that fuels muscle growth during development. In adult muscle, Pax7/3 positive cells, now present as quiescent satellite cells on muscle fibres, are activated on injury and ensure regeneration of the tissue. The behaviour of skeletal muscle stem cells will be discussed in the context of regenerative myogenesis together with the role of the transcription factors, Pitx2 and Pitx3, that we show, are two key regulators of the redox state in adult skeletal muscle stem cells.

L'honoré et al. (2014). Redox regulation by Pitx2 and Pitx3 is critical for foetal myogenesis. Dev Cell, 29, 392-405.

Montarras, D., L'honoré, A., and Buckingham, M. (2013). Lying low but ready action: the quiescent muscle satellite cell.. FEBS Journal; 280, 4036-4050.

Crist, C., Montarras, D., & Buckingham, M. (2012). Muscle satellite cells are primed for myogenesis, but maintain quiescence with sequestration of Myf5 mRNA targeted by microRNA-31 in mRNP granules. Cell Stem Cell, 11, 118-126.

Pallafacchina et al. (2010). An adult tissue-specific stem cell in its niche: a gene profiling analysis of in vivo quiescent and activated muscle satellite cells. Stem Cell Res., 4, 77-91.

Session 1: Stem cells: Genetics / Signalling

The hypoxic stem cell niche: 3D context or autonomous cell behavior?

David Ollitrault, Ph.D., Rosamaria Corerra, Karo Tanaka, Ph.D., Giovanna Marazzi, M.D., and David A. Sassoon, Ph.D.,
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Adult somatic stem cells have been shown to proliferate better and retain stem cell capacity longer when grown in hypoxic conditions. We have examined the stem cell niche in skeletal muscle which consists of the satellite cell that gives rise to new muscle fibers following injury as well as closely associated cells that give rise to fat (FAPs) and vessels. When these cells are isolated from primary muscle tissue and placed into standard culture conditions, they undergo limited rounds of proliferation and then differentiate and/or lose proliferative capacity. When these cells are culture in a 3D context, they maintain their stem cell identity but undergo very limited proliferation. In order to better understand how the stem cell niche is maintained in vivo, we have explored the role of several cell stress inputs (hypoxia, metabolic substrate depletion) and show that a series of cell stress response genes including HIF1alpha, p53, mTOR and PW1/Peg3 are critical for controlling stem cell expansion and proper differentiation while maintaining the stem cell population. These and other data will be discussed.

Session 1: Stem cells: Genetics / Signalling

Plant stem cells: dealing with signal and noise

Ben Scheres[1] , Luca Santuari[1], Gabino Sanchez-Perez[1], Renze Heidstra[1]
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**ben.scheres@wur.nl*

In Arabidopsis roots, the phytohormone auxin and PLETHORA transcription factors control many aspects of developmental progression. Coordination of this progression defines zones of cell division, cell expansion and cell differentiation. We combined experiments and computational modelling to unravel a dynamic interplay between auxin and the PLT proteins. High and prolonged auxin concentrations generate a narrow PLT transcription domain, and a PLT protein gradient extends outward from this domain exploiting growth dilution and cell-to-cell movement.

What are the consequences of this transcription factor gradient with maximum expression values in the stem cell region? We show that different PLT levels define two distinct meristem zones and the expansion/differentiation boundary1. We provide evidence that slow, auxin-dependent PLT thresholds stabilize developmental progression from stem cell to differentiated cell, while rapid PLT-independent auxin action allows fast tropic responses without disturbing the meristem boundary. I will discuss how this mechanism compares to another second noise-filtering mechanism utilizing the RETINOBLASTOMA protein that we found to be important for the control of asymmetric cell division2.

How can a transcription factor gradient encode properties such as stemness and differentiation? We have approached this question by investigating the direct and indirect targets of PLT transcription factors using induced expression and ChIP-seq approaches. The results indicate that division and differentiation control through PLT transcription factors can be separated at the level of induced and repressed target genes.

References
[1] Mähönen et al., Nature 515, 125-129, 2014.
[2] Cruz-Ramirez, A. et al: Cell 150, 1002-1015, 2012

Session 2: Chemistry and Physics of Biomaterials

Mesoscale Polymers and Tertiary Structure Formation

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Keywords: nanoparticle assembly, mesoscale, helices, metamaterials

We present the fabrication and characterization of polymer-nanoparticle mesostructures, which are designed to help transform advanced materials components synthesized at the nanoscale into robust macroscale structures. We call these mesostructures, mesoscale polymers due to their unique configurational capabilities and potential for processing and self-assembly mechanisms similar to polymer molecules. We demonstrate the formation of these mesostructures in the forms of fibrils, helices, and sheets and quantify their mechanics.

Many natural materials demonstrate a unique ability to gently conform to complex topology while maintaining extreme robustness in mechanical strength and resilience. Key to the presentation of such properties is the creation of materials with structural hierarchy, discretized combinations of lengths and angles, and interactions between flexible, yet stiff, components that permit rotational freedom. In nature, this hierarchy begins with the assembly of nanoscale building blocks into fibers and segments, which then turn or assemble into tertiary structures of helices and sheets at the mesoscale. Such tertiary structures are essential for the performance that is realized at the macroscale; however, synthetically relatively few methods exist to create such structures. Furthermore, our understanding of mechanics at these scales has been relatively unexplored.

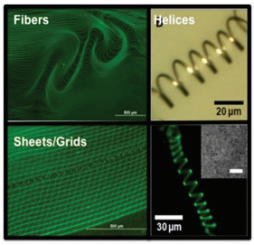

Figure 1 *– Examples of polymer- nanoparticle mesostructures. Top Left: Fibers of tailored CdSe nanoparticles assembled on a water surface. Top Right: Helix of Au nanoparticles in water. Bottom Left: Two dimensional grid of tailored CdSe nanoparticles in water. Bottom Right: Helix of tailored CdSe nanoparticles in water. Inset: TEM micrograph of CdSe nanoparticles within helix ribbon.*

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Approach

We present recently developed methods to assemble tailored nanoparticles and polymers into materials structures, which can be transformed into tertiary structures that possess advantageous attributes. These transformation processes take advantage of the balance between surface energy and elasticity, leading to a robust, geometric approach for complex structure formation. We have initiated a thorough study of the mechanics of these structures, which include ribbons, helices, and 2D grids, which offer promise in applications ranging from flexible electronics to advanced coatings.[1, 2, 3]

To characterize the mechanical properties of these unique mesostructures, we have developed and employed a large variety of physical characterization techniques. We have measured the mechanical properties of individual helices using custom mechanical stretching measurements, consisting of long carbon fiber manipulators and in situ imaging.[1] We have demonstrated that the stiffness of the helical structures can range from the stiffness of a single biomolecule to that of a glass polymer fiber as a function of applied displacement. The elastic modulus of the materials in these structures is typically in the ~GPa range. We have also characterized the reversibility of the helices under flow using microfluidic devices. In addition to measuring the mechanics of individual helices, we have characterized the fiber and grid mesostructures as incorporated onto the surface of or within polymer matrices. In these multi-level composites, the polymer-nanoparticle mesostructures can offer mechanical and functional advantages.

Summary

The methods discussed here offer new opportunities for the creation of structures with dimensions and mechanical properties not currently found in other synthetic materials systems, while also offering clear pathways for scalable manufacturing.

References

[1] J. T. Pham, J. Lawrence, G. M. Grason, T. Emrick, and A. J. Crosby, "Stretching of assembled nanoparticle helical springs.," Phys. Chem. Chem. Phys., vol. 16, no. 22, pp. 10261–6, Jun. 2014.

[2] J. T. Pham, J. Lawrence, D. Y. Lee, G. M. Grason, T. Emrick, and A. J. Crosby, "Highly stretchable nanoparticle helices through geometric asymmetry and surface forces.," Adv. Mater., vol. 25, no. 46, pp. 6703–8, Dec. 2013.

[3] D. Y. Lee, J. T. Pham, J. Lawrence, C. H. Lee, C. Parkos, T. Emrick, and A. J. Crosby, "Macroscopic nanoparticle ribbons and fabrics.," Adv. Mater., vol. 25, no. 9, pp. 1248–53, Mar. 2013.

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The effect of hydroxyapatite nanoparticles on the morphogenesis and properties of collagen I

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Mineralized collagen fibrils constitute the fundamental nanoscale building blocks of many hard tissues. Significant progress has been achieved in the understanding of the fibril formation and mineralization in the process of living bone tissue formation. Manmade collagen matrix composites exhibit range of biological properties pivotal for bone healing and regeneration. No generally accepted relationship between composition and time evolution of mechanical properties of manmade collagen biomaterials has been derived, yet. Here, we report on the kinetics of collagen I (Coll) self-assembly in the presence of hydroxyapatite (HAP) nanoparticles (NPs) and the evolution of mechanical properties of Coll/HAP nanocomposite hydrogels using large amplitude oscillatory shear (LAOS) and viscoelastic measurements. Incresing the strain rate, Coll/HAP *self-assembled* into a range of temporary meso-structures undergoing multiple strain induced structural transformations. At small HAP volume fraction ($v_{f)'}$ the onset of the structural transformations was shifted to larger strains. Above a critical $v_{f'}$ the depletion of the C-termini due to Coll interactions with the OH- groups from HAP greatly restricted the extent of Coll *fibrillogenesis* reducing the stability of the meso-structures. Critical HAP content was determined providing the biomechanical performance close to that of the mineralized collagen fibril and allowing easy scaffold handling during implantation.

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Polymeric Photocurables for artificial biocompatible blood-vessel structures

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Within the EU-project ArtiVasc3D (grant-no263416) polymeric scaffold materials were developed for a vascularized skin model. This model is realized by a three layer buildup of dermal, epidermal and a vascularized fatty tissue layer. The vascularization of biomimetic scaffold systems is still a challenging task in tissue engineering [1].
We introduce material development [2] for rapid prototyping fabrication methods for structuring 3D branched, artificial tube systems.
Photocurable liquid monomers were crosslinked by laser radiation to form cytocompatible, non-degradable or degradable polymers having adjustable mechanical properties. Practical issues such as sewability of polymeric tube systems were of special interest when choosing the monomer compositions for the photocurables. The 3D stereolithography process enabled fabrication of porous tubes and branched tubular structures. Additionally electrospinning technique was used to produce small porous tubes from special designed biocompatible poly(ester-urethane)s (Gugerell et. al.). Biofunctionalization of the tubes was achieved by coating with modified heparin and peptides and subsequent endothelialization [4].

References
[1] Novosel EC, Kleinhans C, Kluger PJ. (2011) *Vascularization is the key challenge in tissue engineering*. Advanced Drug Delivery Reviews, 63(4-5), 300–311.
[2] Meyer W, Engelhardt S, Novosel E, Elling B, Wegener M, Krüger H. (2012) *Soft Polymers for Building up Small and Smallest Blood Supplying Systems by Stereolithography*. Journal of Functional Biomaterials, 3(2), 257–268.
[3] Engelhardt, Sascha, Hu Y, Seiler N, Riester D, Meyer W, Krüger H, et al. (2011) *3D-Microfabrication of Polymer-Protein Hybrid Structures with a Q-Switched Microlaser*. JLMN, 6(1), 54–58.
[4] Birgit Huber, Wolfdietrich Meyer, Esther Novosel, Hartmut Krüger, Annika Wenz, Veronika Schönhaar, Günter Tovar, Petra J. Kluger, Kirsten Borchers, *Cell-adhesive heparin surfaces for endothelialization of photo-curable and cytocompatible polyacrylate elastomers: In preparation*

Session 2: Chemistry and Physics of Biomaterials

Biomaterials and 3D-Printing: Power in Medical Applications

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In recent years a paradigm shift in understanding of human bone formation occurred that starts to change current concepts in tissue engineering bone and cartilage. New discoveries revealed that fundamental steps in bio-mineralization are enzyme-driven, not only during hydroxyapatite deposition, but also during initial bioseeds formation, involving the transient deposition and subsequent transformation of calcium carbonate to calcium phosphate mineral. The principle enzymes mediating these reactions, carbonic anhydrase and alkaline phosphatase, open novel targets for pharmacological intervention of bone diseases like osteoporosis, by applying compounds acting as potential activators of these enzymes. It is expected that these new findings will give an innovation boost for the development of scaffolds for bone repair and reconstruction, which began with the use of bioinert materials, followed by bioactive materials and now leading to functional regenerative tissue-units. These new developments have become possible with the discovery of the morphogenic activity of the bio-inorganic polymers, biocalcit, bio-polyphosphate and biosilica that are formed by a biogenic, enzymatic mechanism, a driving force along with the development of novel rapid-prototyping three-dimensional (3D) printing methods and bioprinting (3D cell printing) techniques that may allow a fabrication of customized implants for patients suffering in bone diseases in the future.

References

[8] W.E.G. Müller, et al., Biomaterials, 2013, 34: 8671
[9] X.H. Wang, et al., Trends Biotechnol., 2014, 32: 441
[10] X.H. Wang, et al., Beilstein J. Nanotechnol., 2014, 5: 610
[11] X.H. Wang, et al., Int. Rev. Cell Mol. Biol., 2014, 313: 27.
[12] W.E.G. Müller, Macromolec. Biosci., 2015, DOI: 10.1002/mabi.201500100

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Organic-inorganic hybrid materials for biomedical applications

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Hybrid organic-inorganic materials (HOIMs) are considered nowadays as potential candidates to play a major role in the development of advanced functional materials, including those for biomedical applications. HOIMs consist of organic and inorganic components intimately mixed, where at least one of the component domains has a dimension ranging from a few tens to several nanometers, and there are chemical bonds (covalent or iono-covalent bonds) between the components [1]. The properties of hybrid materials are not only the sum of the individual contributions of both phases, but the nature and role of their interfaces could be predominant, provided that high precision molecular design toward structural control is carried out at nanometer or even lower sizes. Among inorganic nanoparticles, functionalized POSS are unique nanobuilding blocks that can be used to create a wide variety of hybrid materials, where precise control of nanostructures and properties is required. Condensed silsesquioxanes have the general formula (RSiO1.5)2n, where n is an integer and R can be a large number of substituents including hydrogen, alkyl, alkylaryl, alkenyl, phenyl, halogen and siloxy groups [2-5]. POSS are cytocompatible nanomaterials that can be applied in biomedical area for tissue engineering. Importantly, POSS-based HOIMs can be biofunctionalized
by linking e.g. peptides and growth factors through appropriate surface modification in order to enhance the haemocompatibility of cardiovascular devices made of these materials [5].
In the lecture organic-inorganic hybrid materials containing POSS, designed for biomedical applications, will be presented with special attention paid to polyurethane/POSS hybrid materials.

References

1. P. Gomez-Romero, C. Sanchez (Eds.), Functional Hybrid Materials, Wiley-VCH, Weinheim 2004.
2. K. Pielichowski, J. Njuguna, B. Janowski, J. Pielichowski, Adv. Polym. Sci., 201 (2006) 225.
3. K. Raftopoulos, Ch. Pandis, L. Apekis, P. Pissis, B. Janowski, K. Pielichowski, J.Jaczewska, Polymer, 51 (2010) 709.
4. J.P. Lewicki, K. Pielichowski, P. Tremblot De La Croix, B. Janowski, J.J. Liggat, Polym. Degrad. Stab., 95 (2010) 1099.
5. H. Ghanbari, B.G. Cousins, A.M. Seifalian, Macromol. Rapid Commun., 32 (2011) 1032.

Session 2: Chemistry and Physics of Biomaterials

3D materials based on biopolymers

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3D composites based on collagen, chitosan and/or silk fibroin were prepared by lyophylisation process. The structure of composites was studied by ATR-FTIR technique and was observed by scanning electron microscope. Moreover, miscibility of the blends of two different biopolymers with different compositions in solution were investigated using viscometric method before the lyophylisation process. Mechanical properties of 3D composites made of the blends of two biopolymers were studied and compared with those of single biopolymer sponge. Moreover, inorganic particles were incorporated into 3D biopolymer sponge to get composites with modified biological and mechanical properties.

The properties of 3D biopolymeric materials based on the blends will be discussed as they can be applied in biomedical field an in cosmetic industry.

Session 2: Chemistry and Physics of Biomaterials

New bioceramics and hybrid composites in regenerative medicine

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The progressive ageing of the population and the extended life expectancy are increasingly pushing towards new smart solutions for regeneration of hard connective tissues diseased by trauma, tumours or degenerative pathologies such as osteoarthritis and osteoporosis. Calcium phosphates, particularly hydroxyapatite, are elective biomaterials for bone regeneration; however the conventional synthesis methods fail in achieving ceramic devices exhibiting high chemical, morphological and mechanical mimesis of complex tissues such as bone and osteochondral regions. This is a key aspect determining the ability of the scaffold to trigger the correct cascade of biological events that lead to tissue regeneration, by exposing cells to an adequate array of chemical-physical, structural and morphological signals whose presentation follows precise spatial and temporal patterns, thus triggering specific cell phenotype.
The scaffold design is strongly dependent on the involved anatomical site, so that the approach of material scientist has to be focused on the specific clinical application. The regeneration of critical size bone defects requires scaffolds with osteogenic ability and exhibiting wide open and interconnected macro-porosity permitting scaffold colonization by cells and the development of extensive vascular network. Several techniques are available to produce ceramic scaffolds with wide open interconnected porosity permitting extensive colonization by new bone. However such techniques generally fail in achieving large porous scaffolds with designed structure and nanosized elements, a key factor for exhibiting high osteogenic character and ability of bio-resorption, particularly in long bone regeneration. Therefore material scientists are looking to Nature with ever increasing interest to develop new-generation bio-devices with high bioactivity and hierarchically organized structure.
Similarly, to develop biomimetic scaffolds for regeneration of multifunctional tissues such as osteochondral regions (i.e. composed of bone, mineralized cartilage and hyaline cartilage) or periodontium (i.e. composed of alveolar bone, periodontal ligament and cementum), bio-inspired synthesis processes can be settled, taking inspiration from the natural processes occurring in vivo during formation of new bone tissue. Such cascade of phenomena consisting in self-assembling of Type I collagen and mineralization with nanosized, low crystallinity hydroxyapatite can be reproduced in laboratory, to activate control mechanisms inherent in the organic macromolecule and yield fibrous hybrid constructs reproducing the different tissues of multifunctional anatomical regions.
Highly biomimetic scaffolds with good regenerative properties may be further enhanced by implementation of smart functions, thus enabling personalized and more effective therapies, that is a key issue of increasing relevance. The use of magnetic fields in regenerative medicine is an emerging concept that is still retarded by the use of cytotoxic iron oxide nanoparticles as unique superparamagnetic media enabling smart theranostic applications. In this respect the recent development of a biocompatible and bio-resorbable iron-doped hydroxyapatite nanophase with intrinsic superparamagnetic properties (Fe-HA) may open the way to new perspective in the development of advanced therapies and diagnostic tools. Fe-HA can be used as superparamagnetic nanoparticles exhibiting great potential of binding several bioactive molecules and intense hyperthermia properties, thus being suitable as smart drug delivery system, as killing media for tumor cells and as contrast media for NMR-based analysis. Fe-HA can also be heterogeneously nucleated on self-assembling collagen matrices to give rise to hybrid superparamagnetic and biomimetic scaffolds with enhanced osteogenic character and ability to be remotely activated, on demand, by low magnetic fields.

Session 3: Advanced Instrumentation

Inspecting the role of hormonal crosstalk in the root gravitropic response via in vivo imaging at the sub-cellular resolution

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Changes in the intercellular auxin distribution via prompt relocalization of auxin efflux carriers PIN3 and PIN7 were demonstrated to be necessary for proper root gravitropic response. The role of other phytohormones cytokinins in the control of root gravitropism was proposed, too; however, the experimental evidence was missing. Via detailed study on dynamics of root bending early after gravistimulation, we observed delayed gravitropic response in transgenic lines with depleted endogenous cytokinins *(Pro35S:AtCKX2* and *Pro35S:AtCKX3)* and in cytokinin signaling mutants. Cytokinin perception-deficient mutant ahk3 revealed aberrations in the auxin accumulation in columella cells, suggesting defects in the auxin transport machinery. Accordingly, the membrane and intracellular localization of PIN3 and PIN7 was differentially affected in *AtCKX* overexpression lines and the *PIN7* expression domain was enlarged. Using in vivo real-time imaging of *PIN3-GFP* and *PIN7-GFP* in the *AtCKX3* overexpression and *ahk3* mutant backgrounds we observed wild type-like relocalization of PIN proteins in columella cells during 30 minutes after gravistimulation. Notably, gravity-induced relocalization of PIN7 was faster than that of PIN3 in columella cells. Altogether, our results provide experimental evidence for the role of endogenous cytokinins in controlling the root gravitropic response via differential regulation of the expression and localization of auxin efflux carriers PIN3 and PIN7.

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Session 3: Advanced Instrumentation

Novel roles of phosphoinosites in regulation of chromatin functions

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The eukaryotic cell nucleus is a highly organized and well-choreographed organelle. Based on recent molecular and microscopy data, various dynamic nuclear subcompartments executing specific functions have been described sharing the general feature of lacking the membranes. In a previous study, we revealed a novel important player in nucleolar architecture: we showed that the organization of nucleolus during the cell cycle as well as the transcription of ribosomal genes involve phosphatidylinositol 4,5-bisphosphate (PIP2). Here we describe for the first time additional PIP2-positive nucleoplasmic structures. Using advanced light and electron microscopy techniques combined with multi-immunolabeling approach, we studied these structures which we named PIP2 islets. We show that PIP2 islets are evolutionary conserved lipidic structures surrounded by nucleic acids and protein-containing constituents. PIP2 islets are not chromatin-dependent and do not follow the chromatin rearrangements. At the periphery of the islets, PIP2 co-localizes or is located in immediate vicinity with nascent RNA transcripts as well as with the proteins engaged in Pol II transcription and organization of chromatin. The PIP2 islets are sensitive to RNase treatment, and their enzymatic disruption lowers transcriptional levels. Based on this data, we propose a model in which we speculate that the PIP2 islets play an important role in the organization of nuclear architecture serving as the docking stations for the formation of Pol II transcription complexes.

Key words: nucleus, PIP2, chromatin, Pol II transcription.

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Session 3: Advanced Instrumentation

MicroCT Analysis of Embryos and Soft Tissues

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Understanding animal development depends on accurate visualization of morphology at size scales from whole embryos to ultrastructure, and microscopy methods for life sciences research have been driven by two opposing problems: the demand for ever finer spatial resolutions and the requirement to visualize tissues and structures in situ. For animal embryos and other whole tissue samples, contrast-enhanced microCT imaging is a powerful complement to other 2D and 3D imaging methods. Because tomographic imaging records quantitative spatial and object density information, it naturally generates data suitable for various kinds of modeling and analysis.

My lab is currently involved a wide range of projects in various collaborations and student research. These include refining methods for tissue stabilization, and developing contrasting techniques for tissue- and molecule-specific imaging with microCT. Of particular interest are imaging early cartilage in embryos, and imaging antibody and mRNA probes in whole-mount samples too large and opaque for fluorescence imaging. Related to these goals, we are also working on simple dual-energy scanning procedures using ordinary lab-based microCT systems.

Among our other applications of microCT imaging are systematic studies that are establishing standards for producing and publishing virtual specimens (cybertypes) of museum specimens; biomechanical modeling of functional morphologies based on post-mortem scans; and comparative analyses of organogenesis, particularly limb, eye, and brain development.

Session 3: Advanced Instrumentation

Application of advanced microscopic techniques in molecular biology

Martin Mistrik
Palacký University, Olomouc

The presentation will be aimed on selected advanced fluorescent microscopic techniques involving Structured Illumination (SIM) and Photo-Activation Localization Microscopy (PALM), quantitative high content/throughput fluorescent microscopy and special photo-manipulation techniques such as localized DNA damage induction and FRAP. Various microscopic systems used for our research will be presented and some practical aspects of system calibration and sample preparation will be discussed.

Recommended literature:
Nat Protoc. 2015 Aug;10(8):1248-1263. doi: 10.1038/nprot.2015.083. Epub 2015 Jul 23.
Plant Physiol. 2014 May;165(1):129-48. doi: 10.1104/pp.114.238477. Epub 2014 Mar 31.
Nature. 2015 May 28;521(7553):541-4. doi: 10.1038/nature14328. Epub 2015 Mar 23.
Cell Rep. 2015 Mar 10. pii: S2211-1247(15)00174-6. doi: 10.1016/j.celrep.2015.02.028.
Cell. 2014 Jul 31;158(3):633-46. doi: 10.1016/j.cell.2014.05.046.

Session 3: Advanced Instrumentation

STED 3X – Principle and applications of the next dimension in super-resolution microscopy

Daniel Smeets
Leica Microsystems

Over the past decade several new far-field super-resolution microscopy methods have emerged which overcome the diffraction limit, improving the spatial resolution to the nanometer scale. Stimulated emission depletion (STED) microscopy achieves super-resolution purely optical by engineering the point-spread function through a second red-shifted, donut shaped depletion beam.

STED microscopy provides easy and intuitive access to multi-colour super-resolution as well as live cell imaging. It has and continues to give unprecedented insights to a broad spectrum of research fields including cell and neurobiology.

This presentation explains the basic principle of STED and illustrates the latest development of STED microscopy which expands its benefits to the 3D space.

Application examples of 2D- and 3D-STED with fluorescent proteins as well as standard organic fluorophores will be shown. Furthermore I present recent developments in labelling strategies and discuss their potential for live-cell STED imaging.

Session 3: Advanced Instrumentation

Signalling and mechanics at the human mitotic centromere and kinetochore

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A hallmark of mitosis in mammalian cells is the alignment of chromosomes into a thin metaphase plate. This conserved, reproducible structure probably arises from a coordinated interaction between dynamic microtubule ends, kinetochores, molecular motors, centromeric chromatin, and the spindle checkpoint machinery, but the contributions of these different components has been difficult to discern. Our lab is interested in understanding how this structure forms, and how the individual molecules and structures contribute to the formation of the metaphase plate.

We have used a variety of tools to examine this question—proteomic analysis of chromatin at defined cell cycle states, analysis of post-translational modifications, high spatial and temporal resolution fluorescence imaging in fixed and living cells, and the development of software tools to visualize, manage, and analyse the complex datasets that this work generates.

I will describe our latest results that reveal new regulatory mechanisms that control the assembly and dynamics of mitotic spindles and microtubule-kinetochore attachments. I will focus on the function and roles of Sds22, a PP1-targetting factor, Bod1, a PP2A-B56 inhibitor, and new players in mitotic progression, the prolyl hydroxylases PHD1 and PHD2.

I will also briefly mention our efforts to develop standardized interfaces for biological imaging. This project, the Open Microscopy Environment (http://openmicroscopy.org), is an open source, community-led development effort that delivers data models, file specifications, and data management software for biological microscopy.

Session 3: Advanced Instrumentation

Biomedical Imaging with Synchrotron Radiation: the experience at the SYRMEP beamline of Elettra

G. Tromba
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The SYnchrotron Radiation for MEdical Physics (SYRMEP) beamline at the Elettra light source in Trieste (Italy) is very active in the field of bio-medical imaging.
The beamline is equipped with two imaging stations for users' experiments (with monochromatic and white/pink beam) and a radiological facility for mammography studies on patients.
Biomedical research has been developed following three main directions: clinical mammography, imaging of small animals, high resolution imaging of tissue specimens, organs, biomaterials, etc.
The most used imaging techniques exploiting the spatial coherence of SR source are Propagation Based phase contrast (PB) imaging and Analyzer Based Imaging (ABI), applied either for planar and Computed micro-Tomography (micro-CT) modalities.
For the clinical activity, the first trial of planar PB mammography was completed showing that, compared to conventional mammography, examinations with SR have higher image quality and higher specificity, reducing in particular the number of false negatives. The future step foresees the development of a tomographic protocol that combines the potential of PB imaging with the use of a new CdTe single photon counting detector.
The beamline is well suited for imaging of small animals and some feasibility studies demonstrated also the possibility to face the in-vivo phase. In this framework, examples are the cell tracking procedure developed to follow the marked glioblastoma cells in mice brain and the protocol for morphologic and functional imaging of asthmatic mice.
Micro-CT is extensively applied for high resolution imaging. A challenging application is in regenerative medicine, where it is needed to evaluate the bio-integration of tissue engineering scaffolds in terms of new bone formation and neo-vascularization.
For many of the above mentioned experiments, phase retrieval algorithms are applied to enhance the visibility of the different sample phases prior to the quantitative analysis. New modalities, based on the use of staining procedures, to further increase the image contrast, have been also experienced.
To address the users' request, a new user-friendly software suite has been developed by the SYRMEP team to support absorption and PB microCT studies by offering single distance phase retrieval pre-processing, filtered back projection and other reconstruction algorithms suitable for low-dose or fast CT, where a reduced number of noisy projections is acquired.
The talk will give an overview of the latest achieved results, highlighting the development programs and the main research perspectives.

Session 3: Advanced Instrumentation

3D Imaging at the Nanoscale: unveiling the wonders of nature at the molecular level

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A new frontier exists in unraveling interactive biological and biochemical processes and pathways at the macromolecular level. Of critical importance is the three-dimensional visualization of macromolecular structures and molecular machines in their native functional state. Three techniques play a major role, NMR, XRD and Cryo-TEM.

Nuclear magnetic resonance (NMR) has the capability to study specific protein domains or fragments and their functional role in protein folding and dynamics and in ligand binding whereas X-Ray crystallography (XRD) allows visualizing high-resolution but more static 3D structures, mainly in a monomeric or dimeric state after crystallization. To unravel more physiologically relevant situations however, it is essential to structurally resolve multimeric complexes in their tertiary and quaternary state, how they interact with other complexes and where they are located with cells. Cryo-TEM applications like single particle analysis one can now visualize multimeric complexes at the molecular level. Next, cryo-electron tomography can place these macromolecular structures in their cellular environment by means of selected volume tomography. After identifying the site of interest in a vitrified sample, a vitrified lamellae can be prepared with a focused ion beam and transferred into the cryo-TEM.

Now, finally, a continuum has been reached on all important aspects with regards to resolution and macromolecular scales which will make is possible to unveil the wonders of nature at the molecular level.
We will discuss the future of structural biology based on the latest developments of the FEI workflows and their components.

Session 3: Advanced Instrumentation

Bridging Microscopes: 3D correlative light & scanning electron microscopy of complex biological structures

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Key Words: correlative microscopy, CLEM, CLSM, SEM, FIB-SEM, Array Tomography, membrane imaging, morphome, connectome, structure research

Correlative light and electron microscopy (CLEM) is a powerful tool in life science to combine large-scale volume imaging of cells or tissues by LM with a high-resolution description of their morphology using EM. The combination of 3D microscopy techniques such as CLSM with FIB SEM or serial block face SEM (SBF-SEM) [1] or array tomography (AT) [2] opens up new possibilities to expand morphological context description and analysis into to the third dimension on the nm-scale.
These three approaches to correlate 3D LM data with SEM volume data have been shown to provide a valid alternative to TEM-based serial sectioning approaches [3]. Modern SEM-platforms allow imaging with an x/y resolution of 2 3 nm, and offer the advantage of automation: e.g. routine overnight FIB-SEM volume imaging can cover cross-sections of up to 40×20 μm^2. With a slice thickness of 5-10 nm, these stacks can expand to several 10 µm in the z-axis. FIB-SEM further enables precise, site-specific localisation and milling.
SBF-SEM offers more flexibility when choosing the volume size: theoretically the area to be scanned is limited only by the size of the block face. However, very large scan areas come at the cost of the x/y pixel size or extended scan time (BSE imaging). The slice thickness can be chosen from 15 200 nm to ideally match the biological question. Thus areas from several 100x100µm and several 100µm in depth (volumes: 10000 to $6*10^6$ μm^3) can be recorded [4].
In combination with prior screening of the specimen by confocal LM to identify the ROI, this allows targeted high-resolution 3D imaging of the structure of interest. This approach requires a sample preparation that facilitates the application of both LM and EM on a bulk specimen. To make this possible we have adapted protocols for freeze-substitution after high-pressure freezing, or chemical fixation and embedding in resin to include fluorophores in the samples [5].
Thus fluorescently labeled, resin-embedded specimens can also be used for AT. Here, ribbons of serial sections are loaded onto glass-slides, enabling even larger ROIs to be investigated by wide-field LM and subsequently by SEM. With the introduction of special surface coatings such as ITO these glass supports remain transparent for LM, but become highly conductive for SEM investigations. Such ribbons can be stained for histology (e.g. Toluidine blue or H&E) or for fluorescent immuno-labeling to specifically identify a ROI by F/LM prior to HR-SEM imaging. If needed, the sections can also be post-stained with uranyl acetate for (FIB)SEM-imaging.

Software solutions are available to facilitate the relocation of the ROI by a marker-based calibration of the sample in both LM and (FIB-)SEM. And automated recording of 3D volumes is possible for all three described methods. However, the FIB-SEM approach can achieve the better z-resolution, whereas the z-resolution for AT and SBF-SEM is limited by the sectioning process. FIB-SEM can therefore record isotropic voxels, which is an advantage when it comes to 3D reconstruction and modeling. SBF-SEM and AT on the other hand provide a much larger field of view. And AT even enables specific fluorescence labelling to identify the ROI combined with immune-gold labelling for HR-SEM. For SBF-SEM the ROI has to be defined using the information which can be obtained from the surface of the block face, either by LM or SEM. Furthermore, FIB-SEM and SBF-SEM are destructive methods, while the AT-ribbons can be stored and re-investigated ad libitum [6].
Resulting 3D datasets from LM and EM imaging have very different resolutions but can be merged in-silico to 3D models. By combining with other imaging modalities (e.g Xray-CT) one can reconstruct the structural hierarchical scaffold and hence better understand the complexity of living systems. Further transforming these structures into a visual perception space or haptic perception space by 3D printing the nano-world becomes better (comprehensive) accessible also for the layman. The method of choice has to be determined anew for every project, according the sample type, the size of the structure or the volume of interest and the desired imaging resolution in x,y, and z.

Literature:
[1] W Denk *et al*, PLoS Biol 2 (2004), p. e329.; [2] KD Micheva *et al*, Neuron 55 (2007), p. 25.
[3] MS Lucas *et al*, Methods Mol. Biol. 1117 (2014), p. 593.;[4] CJ Peddie *et al*, Micron (2014), p. epub ahead of print.; [5] SS Biel et al, J. Microsc. 212 (2003), p. 91.; [6] MS Lucas *et al*, Methods Cell Biol. 111 (2012), p. 325.

Further Readings on correlative 3D imaging applications:
Book on CLEM:
„*Correlative Light and Electron Microscopy*" Vol 111; "*Methods in Cell Biology*" 01/2012;
Some protocols in: „*Electron Microscopy*" in Methods in molecular biology Vol 1117 (Kuo, John - Ed.) 01/2014

Atom-Probe and STEM: Arslan, I., Marquis E. A., Homer M., Hekmaty M. A., Bartelt N. C. "*Towards better 3-D reconstructions by combining electron tomography and atom-probe tomography*; Ultramicroscopy 108, 1579-1585 (2008)
X-ray Tomo and FIB/EM: Dierolf M., Menzel A., Thibault P., Scheider P., Kewish C. M., Wepf R., Bunk O., Pfeiffer R.; "*Ptychographyic X-ray computed tomography at the nanoscale*"; Nature 467, 436-440 and Guizar-Sicariros M., Diaz A., Holler M., Lucas M.S., Menzel A., Wepf R. & Bunk O. "*Phase tomography from x-ray coherent diffractive imaging projections, Optics Express*, 19 (22) (2011)
LM, X-Ray, EM etc.: Mitchell S., Michels N-L., Kunze K. and Perez-ramirez J.; "*Visualization of hierarchically structured zeolites bodies from macro to nano length scales*; Nature Chemistry 4, (2012)

Session 4: Cell-Biomaterial Interface

Defined 3D microenvironments boost induction of pluripotent stem cells

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In the last decade cell reprogramming technology led to the groundbreaking discovery of the induced pluripotent stem cells (iPSCs). Numerous approaches have been explored to improve the original protocol based on a twodimensional cell culture system. Surprisingly, nothing is known about the effect of a more biologically faithful three-dimensional (3D) environment on somatic cell reprogramming.

Therefore, our goal was to apply 3D reprogramming protocols to improve the generation of iPSCs. To this aim, we modulated microenvironmental stiffness, degradability and biochemical composition, to identify the role of biophysical effectors in promoting cell reprogramming. Our data showed that the physical cell confinement imposed by the 3D microenvironment boosts generation of mouse iPSCs via accelerated mesenchymal-to-epithelial transition and increased epigenetic remodeling. Thus, 3D microenvironmental signals can act synergistically with reprogramming transcription factors to increase somatic plasticity. These findings open up new avenues for cell reprogramming and regenerative medicine.

Session 4: Cell-Biomaterial Interface

Hierarchical open and closed polymeric devices for regenerative medicine

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The development of devices for tissue engineering and regenerative medicine are often inspired by the composition and complexity of native structural tissues. At the lowest level of such organization, one should select the adequate biomaterials to be used as the building block of the structure that will support cells and control their behaviour towards the production of new tissue. We have been proposing the use of multilayered based arrangements that could be then integrated in more complex macroscopic devices, often exhibiting a hierarchical organization. Such nanostructured multilayered films may be assembled using oppositely charged polyelectrolytes through the layer-by-layer technology. Using adequate templates, non-flat coatings can be fabricated with tuned compositions along the build-up assembly. This enables the production of very well controlled multifunctional and structural devices using mild processing conditions that could be useful in biomedicine, including in tissue engineering. In particular we have been interested in developing more complex/hierarchical structures that could fulfil specific requirements in such kind of applications.
Examples of nano-stratified surfaces with tuned characteristics are presented, using polysaccharides or synthetic biomimetic macromolecules. Methodologies developed in our group will be exemplified, permitting the production of: (i) 3-dimensional nanostructured scaffolds for tissue engineering, enabling the support of cells in open porous structures; and (ii) multi-scale spherical objects to encapsulate cells, acting as "living" injectable or implantable devices.

Session 4: Cell-Biomaterial Interface

Advanced biomaterials & scaffold–based systems for the delivery of therapeutics: new frontiers in regenerating organs & tissues

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Tissue engineering typically uses a combination of biomaterial scaffolds, cells and signalling mechanisms (such as growth factors or mechanical stimuli) to restore the function of damaged or degenerated tissue. The research carried out in our laboratory investigates each of these three areas with target applications in bone, cartilage, cardiovascular, respiratory, neural and corneal tissues. Recent work has led to the development of a series of porous collagen-based scaffolds with the tailored composition, pore structure and stiffness to promote regeneration of individual tissues. A number of these technologies have been patented resulting in the formation of a spin out company, SurgaColl Technologies, which has brought a number of innovative degradable regenerative technologies for bone and cartilage repair to the market. In the cellular area, we are investigating the therapeutic potential of stem cells in combination with these scaffolds and we have a particular interest in using biophysical stimuli (applied by bioreactors or controlled by scaffold stiffness) to regulate stem cell differentiation. Ongoing research increasingly focuses on the use of these scaffolds as therapeutic bioactive platforms for the controlled delivery of growth factors (eg: [1] Lopez Noriega et al., 2014) or as gene-activated matrices to promote enhanced tissue repair (eg: [2] Curtin et al. 2012). In the former area, we often use microparticle-based approaches to control the release of recombinant proteins [3] while in latter area we focus on the development of non-viral nano-particulate systems for gene delivery. By using tailored specific non-vectors embedded with the scaffolds including PEI [4, 5], chitosan [6] and nano-hydroxyapatite [7, 8], we can achieve a sustained but ultimately transient gene expression profile while still achieving transfection efficiencies in stem cells sufficient to elicit a therapeutic response. These systems have now been applied for delivery of plasmid DNA, microRNA mimics and inhibitors and siRNA nanotherapeutics [9]. This presentation will provide an overview of this ongoing research with particular focus on scaffold-based delivery of therapeutic genes, microRNAs [8] for enhancing bone and cartilage regeneration.

References

[1] Lopez-Noriega A, Ruiz-Hernandez E, Quinlan E, Storm G, Hennink WE, O'Brien FJ. Thermally triggered release of a pro-osteogenic peptide from a functionalized collagen-based scaffold using thermosensitive liposomes. Journal of controlled release : official journal of the Controlled Release Society. 2014;187:158-66.
[2] Curtin CM, Cunniffe GM, Lyons FG, Bessho K, Dickson GR, Duffy GP, et al. Innovative collagen nano-hydroxyapatite scaffolds offer a highly efficient non-viral gene delivery platform for stem cell-mediated bone formation. Advanced Materials. 2012;24:749-54.
[3] Quinlan E, López-Noriega A, Thompson EM, O'Brien FJ. Development of collagen-hydroxyapatite scaffolds incorporating PLGA and alginate microparticles for the controlled delivery of rhBMP-2 for bone tissue engineering. Journal of Controlled Release 2015;(Accepted for publication).
[4] Tierney EG, McSorley K, Hastings CL, Cryan S-A, O'Brien T, Murphy MJ, et al. High levels of ephrinB2 over-expression increases the osteogenic differentiation of human mesenchymal stem cells and promotes enhanced cell mediated mineralisation in a polyethyleneimine-ephrinB2 gene-activated matrix. Journal of Controlled Release. 2013;165:173-82.
[5] Tierney EG, Duffy GP, Hibbitts AJ, Cryan S-A, O'Brien FJ. The development of non-viral gene-activated matrices for bone regeneration using polyethyleneimine (PEI) and collagen-based scaffolds. Journal of Controlled Release. 2012;158:304-11.
[6] Raftery R, O'Brien FJ, Cryan SA. Chitosan for gene delivery and orthopedic tissue engineering applications. Molecules. 2013;18:5611-47.
[7] Curtin CM, Tierney EG, McSorley K, Cryan SA, Duffy GP, O'Brien FJ. Combinatorial gene therapy accelerates bone regeneration: non-viral dual delivery of VEGF and BMP2 in a collagen-nanohydroxyapatite scaffold. Advanced healthcare materials. 2015;4:223-7.
[8] Castano IM, Curtin CM, Shaw G, Murphy JM, Duffy GP, O'Brien FJ. A novel collagen-nanohydroxyapatite microRNA-activated scaffold for tissue engineering applications capable of efficient delivery of both miR-mimics and antagomiRs to human mesenchymal stem cells. Journal of controlled release : official journal of the Controlled Release Society. 2015;200:42-51.
[9] Fitzgerald KA, Guo J, Tierney EG, Curtin CM, Malhotra M, Darcy R, et al. The use of collagen-based scaffolds to simulate prostate cancer bone metastases with potential for evaluating delivery of nanoparticulate gene therapeutics. Biomaterials. 2015;66:53-66.

Session 4: Cell-Biomaterial Interface

Mechanobiology in the Vascular Development and Central Nervous System

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Biological processes are tightly regulated through complex signal networks that are triggered and mediated in large part by soluble cues. Efforts to date to control cellular processes (cell differentiation, cell polarity, tissue morphogenesis) therefore have focused on elucidating the role of soluble signals such as morphogens and mitogens in controlling cell fate and cellular processes. However, we have rather limited insights into the role of non-biological cues emanating from the cellular microenvironment on cellular and signaling homeostasis. Over the past five years our laboratory has unraveled the role of biophysical characteristics of the cellular microenvironment (stochastic roughness, stiffness) on cellular organization and cell function and identified a clear role for mechanobiology in vascular development and central nervous system organization, function and degeneration (1, 2).

References

[1] Forget, Shastri, et al, Polysaccharide hydrogels with tunable stiffness and provasculogenic properties via α-helix to β-sheet switch in secondary structure, (2013) PNAS, DOI: 10.1073/pnas.1222880110
[2] Blumenthal, Hermanson, Heimrich, and Shastri, Stochastic Nanoroughness Modulates Neuron-Astrocyte Interactions and Function via Mechanosensing Cation Channels, (2014) PNAS, DOI: 10.1073/pnas.1412740111

Session 4: Cell-Biomaterial Interface

Biofunctional Polymer Matrices for Stem Cell Bioengineering

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Deciphering the role of extracellular matrices (ECM) in stem cell microenvironments is often hampered by the lack of methods for recapitulating defined and effective ECM assemblies in vitro. Therefore, we have recently developed protocols for the reliable anchorage of native cell-secreted ECM to culture carriers. The approach was validated in the culture of human mesenchymal (MSC) and hematopoietic stem and progenitor cells (HSPC) in vitro, demonstrating the unique ability of the cell-secreted matrices to modulate expansion and differentiation of the bone marrow stem cells [1]. Furthermore, we have introduced a rational design strategy for ECM-inspired hydrogel networks consisting of multi-armed poly(ethylene glycol), sulfated glycosaminoglycans and functional peptide units to allow for the decoupling of biochemical and mechanical matrix properties. Using this approach, different combinations of matrix characteristics were identified to effectively stimulate therapeutically relevant cell fate decisions [2, 3].

References

[1] Prewitz, M.; Seib, F.P.; v. Bonin, M.; Friedrichs, J.; Stißel, A.; Niehage, C.; Müller, K.; Anastassiadis, K.; Waskow, C.; Hoflack, B.; Bornhäuser, M.; Werner, C.: Tightly anchored tissue-mimetic matrices as instructive stem cell microenvironments. Nature Methods 10 (2013) 788

[2] Freudenberg, U.; Sommer, J.-U.; Levental, K.; Welzel, P.; Zieris, A. ; Chwalek, K. ; Schneider, K. ; Prokoph, S. ; Prewitz, M. ; Dockhorn, R. ; Werner, C.: Using mean field theory to guide biofunctional materials design. Advanced Functional Materials 22 (2012) 1391

[3] Tsurkan, M.; Chwalek, K.; Prokoph, S.; Zieris, A.; Levental, K.R.; Freudenberg, U.; Werner, C.: Defined polymer-peptide conjugates to form cell-instructive star-PEG-heparin matrices in situ. Advanced Materials 25 (2013) 2606

Session 5: 3D Modelling

How do epithelial cells invade, disseminate, and metastasize?

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Most cancers arise in epithelial cells and most cancer deaths are attributable to metastasis. These facts are surprising, as epithelial tissues are characterized by their intercellular adhesion and are non-motile during homeostasis. The epithelial cell therefore seems an unlikely starting point for malignancy. Yet, the prevalence of carcinomas argues that there are efficient ways to initiate epithelial programs of migration and proliferation.
A major barrier to understanding metastasis is the relative inaccessibility of the process in vivo. It occurs over years, deep within the body. To overcome this barrier, we developed 3D culture assays to enable us to study epithelial development and malignant progression in real-time. These studies revealed that mammary development involves transient stratification and loss of apico-basal polarity, both induced by an asymmetric cell division. The resulting highly migratory cells remain confined within the epithelium under normal conditions. However, alterations to either the extracellular matrix or the signaling state of the migratory cell are sufficient to induce escape, or dissemination, of cells out of the epithelial group. We next sought to understand the cellular basis of metastasis and applied our assays to discover a conserved molecular program expressed in the most invasive cells within breast tumors. These gene expression changes represented a shift in epithelial differentiation from luminal to mixed luminal-basal and correlated with an increase in migratory and matrix-remodeling behaviors. We are currently focused on understanding the programs driving dissemination and on the nature of the cell (or cells) that serves as the seed for distant metastasis.

Session 5: 3D Modelling

Molecular Geometry and Its Operating System: A New Computational Paradigm for Understanding the Structure of Atomic Arrangements

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The geometry of atomic arrangement underpins the structural understanding in many fields. In biology, the structure of biomolecules determines their function. In chemistry, physics, and materials science, the properties of inorganic molecules result from the geometrical arrangement of their atoms. However, a general framework of mathematical/computational theory for the geometry of atomic arrangement does not exist. Here we present ``Molecular Geometry (MG)" as such a theoretical framework and ``MG Operating System (MGOS)" which consists of callable functions based on the Voronoi diagram of spherical atoms that implements the MG theory. MG facilitates simpler modeling of molecular structure problem in geometry-centric principle and the MGOS functions can be conveniently embedded in application programs for the efficient and accurate solution of geometric queries involving atomic arrangements. MG, accompanied by MGOS, will allow researchers to focus more on their primary research issues by freeing them from the time-consuming and error-prone tasks of developing and implementing highly sophisticated and complex geometry algorithms for molecular structure studies. The impact of math libraries of general purpose high-level programming languages to science and engineering is an appropriate metaphor of that of MGOS, which is currently, freely available at Voronoi Diagram Research Center, to the worlds consisting of spherical entities including atomic arrangements.

References

[13] J.-K. Kim, Y. Cho, M. Lee, R. Laskowski, S.-E. Ryu, K. Sugihara, D.-S. Kim, BetaCavityWeb: a webserver for molecular voids and channels, Nucleic Acids Research, 43(W1), 2015.
[14] D.-S. Kim, Y. Cho, K. Sugihara, J. Ryu, and D. Kim, Three-dimensional Beta-shapes and Beta-complexes via Quasi-triangulation, Computer-Aided Design, Vol. 42, Issue 10, 2010.
[15] D.-S. Kim, Y. Cho, and K. Sugihara, Quasi-worlds and Quasi-operators on Quasi-triangulations, Computer-Aided Design, Vol. 42, Issue 10, 2010.
[16] D.-S. Kim, Y. Cho, and D. Kim, Euclidean Voronoi diagram of 3D balls and its computation via tracing edges, Comp. Aided Design, Vol. 37, No. 13, 2005.
[17] D.-S. Kim, D. Kim, and K. Sugihara, Voronoi Diagram of a circle set from Voronoi Diagram of a point set: II. Geometry, Computer Aided Geometric Design, Vol. 18, 2001.

Session 5: 3D Modelling

How computer simulations help understand the biomedical world

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Society's growing need to understand and respond to problems at the biochemical and molecular levels has triggered rapidly developing powerful tools in order to analyze and model complex processes and systems in the areas of human health, medicine, biochemistry and molecular biology. In this talk we briefly focus on three distinct cases, DNA translocation dynamics through a nanopore [1], periciliary semiflexible (bio)polymer brush behavior under load [2], and genome ejection from virus capsids [3], which have been modeled and studied intensively in recent years. Molecular Dynamics and Monte Carlo simulations are shown to provide valuable insight and elucidate the mechanisms that drive such processes.

References

[1] SINGLE-POLYMER DYNAMICS UNDER CONSTRAINTS: SCALING THEORY AND COMPUTER EXPERIMENT, Andrey Milchev, J. Phys.: Condens. Matter – Topic Review, 23, 103101 (2011)
[2] UNCONVENTIONAL ORDERING BEHAVIOR OF SEMI-FLEXIBLE POLYMERS IN DENSE BRUSHES UNDER COMPRESSION, A. Milchev and K. Binder, Soft Matter, 2014, DOI: 10.1039/C3SM53133C, 10, 3783 (2014)
[3] EJECTION OF A POLYMER CHAIN FROM A NANOPORE: THEORY & COMPUTER EXPERIMENT, A. Milchev, L. Klushin, A. Skvortsov, K. Binder, Macromolecules, 43, 6877(2010).

Session 5: 3D Modelling

Passive endocytosis mediated by ligand-receptor interactions

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Cellular uptake is a crucial process for nanotoxicity and drug delivery. One of the general uptake paths is a receptor-mediated endocytosis, where ligand coated nanoparticles can cross a phospholipid membrane due to the ligand-receptor interaction. However, the conditions under which such passive endocytosis, i.e. not ATP driven, occurs are not well understood.
Using Molecular Dynamics simulations with coarse grained model we investigated the conditions of the passive uptake and consequential cytosol escape of the ligand-coated nanoparticles with varying size, shape, ligand coverage and distribution, and receptor-binding strength.1,2 We found that larger spherical particles undergo endocytosis easier than smaller ones and sharp edges on nanoparticles can hinder the uptake.1 Moreover, we showed that the fastest in uptake are the nanoparticles with the most homogeneous ligand distribution, where ligands are spread almost evenly on the surface. The slowest were the nanoparticles with freely diffusing ligands, which became very inhomogeneous upon interaction with the membrane.2 Those even prevented the uptake unless almost the whole (about 80 %) of the nanoparticle was covered. We were able to rationalize the above findings in terms of elastic theory and competition between membrane bending rigidity and ligand-receptor adhesive energy.
Our results demonstrate how the nanoparticle uptake rate can be influenced the nanoparticle properties. This insight shall to be used for rational redesign of the nanoparticles used in nanomedicine.

References
[1] Vácha, R.; Martinez-Veracoechea, F.J.; Frenkel, D.: Receptor-Mediated Endocytosis of Nanoparticles of Various Shapes. Nano Letters 2011, 11 (12), 5391-5395
[2] Schubertová, V.; Martinez-Veracoechea, F.J.; Vácha, R.: Influence of ligand distribution on uptake efficiency. Soft Matter 2015, 11, 2726-2730,

Session 5: 3D Modelling

ENGINEERING OF 3D-TISSUES

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Tissue engineering is an emerging multidisciplinary field involving biology, medicine and engineering. Depending to the biomedical application, our group uses cell lines or primary (stem) cells combined with biological-based matrices that are specifically adapted to mimic the In-vivo microenvironment of selected tissues. Thus, we have previously established a process for manufacturing collagen matrices with a persisting blood circulation system (BioVaSc® technology). The matrices are based on decellularized organs such as porcine intestine, with intact blood vessel structures allowing us to generate and maintain 3D tissue structures. Based on the BioVaSc® technology, In-vitro models of various organ systems such as the human gastrointestinal, respiratory tracts or skin have been established and functionally tested. Further, it has been shown that mechanical parameters such as media flow, rotation, tension, extension or pulsation stress are critical for the development of 3D bioartificial tissues.

Our 3D tissue equivalents allow us to study different steps of tumor development, drug absorption or infections without the need for testings in animals.

Abstracts of Participants

1 Removal of Pb^{+2} from aqueous medium using nanosilica prepared from solid waste biomaterial

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Environmental pollution with lead still one of the major problems takes attentions from researchers due to their increased discharge and its stability. Exposure to lead causes impaired blood synthesis, hypertension, severe stomach ache, brain, kidney damage and even can cause miscarriage in pregnant women. A nanosilica powder was obtained by heat treatment of rice husk ash (RHA) following the sol-gel method without adding any chemical surfactant. Nitrogen adsorption at 77K showed a surface area of 665 m^2/g, total pore volume 0.646 mL/g and pore radius 1.87 nm. Transmission electron microscopy (TEM) micrographs and particle size showed that about 69% of the particle size was in the range of 20-25 nm. X-ray diffraction (XRD) showed the amorphous nature of the synthesized nanosilica. TEM provided a value of the crystallite size around 26 nm. Adsorption of lead cations on the prepared sample showed linear Langmuir plot (R2 = 0.996) with maximum adsorption capacity of 102.8 mg/g. The kinetic adsorption followed pseudo-second order kinetics. The thermodynamic parameters have been determined and indicated spontaneous endothermic process. Pure water and diluted nitric acid solution were used to study the recovery of the adsorbed lead indicating the ability of acidified medium to recover about 98% of lead.

2 Use of microfluidics to determine vasculature behavior during inflammatory response

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Tissue inflammation activates endothelium in order to increase adhesiveness for immune cells. This results in rolling and firm adhesion as well as rapid extravasation of leukocytes and their migration to the inflamed locus. We focused on phenotype of endothelial cells and their response to inflammatory stimuli in connection with neutrophil adhesion. This could be studied by means of intravital microscopy which is however very demanding. That is why we checked if a microfluidic system is applicable for this purpose. Endothelial cells were grown under flow conditions. They showed elongated shape and tend to arrange along with the flow. The activation of endothelial cells was carried out by means of tumour necrosis factor alpha (TNFα) or myeloperoxidase (MPO). The suspension of neutrophils was flown over the endothelial cells and the rolling and firm adhesion was monitored. Pre-treatment of endothelial cells with TNFα or MPO induced neutrophil adhesion. The microfluidic system were found to be suitable to study pro-inflammatory effect of MPO and TNFα on vascular endothelium.

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3 Inhibition of miR-145 in primary human fibroblasts facilitates reprogramming to induced pluripotent stem cells.

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Cell reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) requires comprehensive rearrangement of cellular signalling pathways and molecular profiles, including the microRNA profile. MicroRNA (miRNA) molecules are short, non-coding RNA molecules that play crucial active role in many cellular processes by post-transcriptional regulation of specific mRNA. Although some miRNAs have been shown to play an important role in various stages of reprogramming, our understanding of how miRNAs regulate this process remains elusive.

We undertook a miRNA expression profile to identify candidate miRNAs whose expression is significantly changed during reprogramming of fibroblasts to iPSCs. This analysis indicated that miR-145 is highly expressed in human fibroblasts (hFs) and its expression is rapidly down-regulated at early stage of the reprogramming process to iPSCs. In order to study the role of miR-145 in detail, we inhibited miR-145 in hFs. Inhibition of miR-145 led to the induction of "cellular plasticity" demonstrated by: I) change of cellular morphology from long-spindled fibroblast shape to a short-spindle epithelial morphology; II) upregulation of pluripotency genes including *KLF4, SOX2, c-MYC,* III) down-regulation of miRNA Let-7b, and IV) down-regulation of mesenchymal genes. Furthermore, inhibition of miR-145 led also to significantly higher reprogramming efficiency to iPSCs in the presence of reprogramming factors, indicating an important role of miR-145 in cell reprogramming.

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4 Optimized protocol for high-throughput toxicity testing in 3D spheroid cultures

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Three-dimensional (3D) cell cultures are becoming more frequently used in vitro models in toxicology, as they mimic more closely in vivo tissue microenvironment, have a better cytoarchitecture and biological relevance when compared to the 2D monolayer cell cultures. 3D cell spheroids can be formed using different scaffold-free cell culture techniques, such as hanging drop method, simulated microgravity systems, perfusion bioreactors, low attachment surfaces or nonadhesive hydrogels.

In our laboratory, we adapted a commercially available technique based on micromolded nonadhesive hydrogels (3D Petri Dish™), and further optimized the experimental protocol to allow automated acquisition of spheroid images from 24-well plates. The experimental workflow was complemented by our in-house developed ImageJ script for automatic high-throughput processing of the acquired images and analyses of spheroid counts, size and shape. We are currently using this simple, cost-effective and (semi) high-throughput method for studying hepatotoxic and hepatocarcinogenic effects of emerging environmental contaminants, such as cyanobacterial toxins microcystin-LR and cylindrospermopsin, in liver progenitor, stem and cancer cell lines (WB-F344, WB-ras, HL1hT1, HepG2).

In summary, we successfully optimized and introduced a novel protocol tested with a number of cell lines that allow simple, rapid and affordable preparation and (semi) high-throughput evaluation of biological changes in 3D spheroid cell cultures.

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5 Use of microfluidics to achieve native phenotype of endothelial cells

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Vasculature is a complex system composed of many cell types organized in a specific manner to fulfill its physiological function. Our main focus is the inner layer of endothelial cells. They are indispensable for maintenance of vascular tone and immune response. The native phenotype of these cells is characterized by elongated shape, organization along with the blood flow, tight contacts among them and remarkable glycocalyx layer at the luminal surface. Such phenotype is however suppressed under standard static in vitro cultivation. In order to get native phenotype endothelial cells (HUVEC) were cultivated under shear stress in a flow through system operated by IBIDI pump. Flow conditions resulted in elongation of cells and longitudinal orientation with flow. Further, elevated expression of glycocalyx-related genes was detected using RT PCR.

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6 Computational study of lectin PA-IIL

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Pseudomonas aeruginosa is a human opportunistic pathogen. It can cause infection of immunocompromised people or people suffering from cystic fibrosis, which is often fatal. Adhesion of the bacteria on human cell surface is mediated by interaction of bacterial lectins with cellular surface carbohydrates. PA-IIL is Pseudomonas aeruginosa tetrameric lectin, which contains two calcium ions in each binding site and recognizes fucosylated oligosaccharides [1]. Saturation of bacterial surface lectins by specially designed compounds might prevent adhesion to host tissues and thus suppress the infection.

In this work, binding affinities of various monosaccharide ligands to the active site of PA-IIL were analyzed by accurate quantum chemical calculations as this information is crucial for rational design of new lectin inhibitors. Due to the system size, the hybrid QM/MM approach was used employing the AMBER force field [2] for description of the MM zone and DFT for description of the QM zone. In the final stage DLPNO-CCSD(T)[3] in the CBS limit was used for calculation of binding affinities.

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[1] Sabin, C.; Mitchell, E. P.; Pokorná, M.; Gautier, C.; Utille, J.P.; Wimmerová, M.; Imberty, A. FEBS *Letters* **2006**, 580 *(3)*, 982.
[2] Case, D.; Babin, V.; Berryman, J.; Betz, R.; Cai, Q.; Cerutti, D.; Cheatham Iii, T.; Darden, T.; Duke, R.; Gohlke, H.; others. Amber 2015; University of California: San Francisco, **2015**.
[3] Neese, F. *WIREs Comput Mol Sci* **2012**, 2 (1), 73.

7 Effects of polycyclic aromatic hydrocarbons in human bronchial epithelial cell line

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Gap junctional intercellular communication (GJIC) is the central mechanism responsible for maintenance of tissue homeostasis. Dysregulation of GJIC by chemical exposure or oncogenic events has been associated with different pathologies, including cancer. Since polycyclic aromatic hydrocarbons (PAHs) represent air pollutants known to elicit tumor promoting and carcinogenic effects in human respiratory system, we investigated effects of PAHs on GJIC in a human bronchial epithelial cell line HBE1. GJIC was rapidly (within 30 min) inhibited by lower molecular weight PAHs with a bay- or bay-like structural region, such as fluorene, fluoranthene, 1-methylanthracene, 9-methylanthracene and pyrene. Conversely, lower molecular weight PAHs without bay- or bay-like region (2-methylanthracene, anthracene) or higher molecular weight PAHs (chrysene) did not inhibit GJIC. These results correspond to the previous findings in rat liver epithelial cells, which indicated that nongenotoxic or weakly genotoxic but highly prevalent lower molecular weight PAHs dysregulate GJIC, the key homeostatic mechanism, which might contribute to their tumor promoting effects. However, these effects on GJIC were evaluated in 2D cell cultures, whose in vivo relevance is limited by the lack of cell polarity, ability to differentiate, cell-cell and cell-extracellular matrix interactions. Therefore, our current research focuses on the use of 3D cell cultures of HBE1 cells to correlate the structure-dependent effects of PAHs on GJIC observed in 2D cultures with effects of PAHs in 3D structures.

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8 Novel Gas Chromatography Procedure for Kinetics Study of D,L-lactide and Glykolide Copolymerization

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Biodegradable thermosensitive ABA triblock copolymer (PLGA-PEG-PLGA) is used in medicine as injectable drug delivery carrier. Determination of monomer conversion is one of the most important experimental variables in kinetics study. The most practical way to measure conversion in a reaction is gravimetrically. However, this method acquires the sample preparation (purification the original mixture prior the weighting) at which the loss of monomer. Conversion may also be obtained by gas chromatography (GC). Nevertheless, due to the similar sublimation temperature of both D,L-lactide and glycolide monomers, it is difficult to use the GC for their separation and conversion evaluation. The GC separation is especially affected by the type of stationary phase (SP), the type of mobile phase (carrier gas) and the temperature. In this work, GC procedure has been optimized in terms of choosing right type of SP and carrier gas, suitable solvent and internal standard and also set-up certain initial and maximal column temperature together with suitable heating rate, injection temperature and flow rate of carrier gas. Finally, the novel procedure of GC has been developed to study the kinetics of D,L-lactide and glycolide copolymerization in order to set-up optimal copolymerization conditions. Preferably, the new GC procedure permits a direct quantification of the residual monomer concentration without any purification of sample prior the GC analysis, the procedure is simple, fast and accurate.

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9 Adherence and growth of cariogenic biofilms on dental dimethacrylate-based resin substrates

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Dental plaque is a complex microbial biofilm consisting of hundreds of bacterial species embedded within a self-produced matrix of extracellular polymeric substance (EPS). In the current dental practice, resin composite restorative materials with the matrix based on dimethacrylate monomers are often the first choice for many convenient properties. However, the dental plaque deposition is provably influenced by an introduction of the novel artificial surface to the oral environment. This may lead to the proliferation of cariogenic bacterial species and eventual origination of secondary caries, especially in the area of tooth-restoration interface.

The quantities of *Streptococcus mutans* biofilms on commonly used dimethacrylate resin substrates were evaluated. This included the systems based on aromatic bis-GMA and bis-EMA and aliphatic TEGDMA monomers and their copolymers. Surface free energy (SFE) of each system was determined via the contact angle measurement. Biofilms were quantified by metabolic viability tests (reduction of XTT and reazurin), showing statistically significant differences between tested materials.

The amount of *S. mutans* biofilm increased from TEGDMA homo-polymer along with the increasing content of either bis-GMA or bis-EMA. Notably, regardless of the great differences between relatively low surface free energy of bis-EMA and high surface energy of bis-GMA associated with the presence of –OH groups. This could be explained by π-interactions between polymer aromatic backbone, and, among others, the dextran polysaccharide produced by S. mutans as an essential component of EPS or other saccharides associated with the surface of the microbial cells.

10 Corrosion Test of Magnesium in Simulated Physiological Conditions: Imaging analysis using X-ray Computed Micro-Tomography Platform

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In biomaterials the accurate assessment of magnesium degradation is crucial for the development of safe therapeutic approaches. Several techniques have been developed to evaluate the corrosion rate of magnesium. Among them X-ray computed micro-tomography is the most recent method used. This work validates the potential of this method to discern between different corrosion rates in different surfaces of cold-rolled magnesium samples, representing a real advantage in respect to other methodologies that only evaluate the global corrosion. Results show that magnesium corrodes faster in plastic deformation zones and that the passivation properties of the corrosion products vary between different simulated physiological fluids.

References

[1] N.T. Kirkland, N. Birbilis, Magnesium Biomaterials: Design, Testing, and Best Practice, firsth ed., Springer International Publishing, Switzerland, 2014.
[2] X. Gu, Y. Zheng, Y. Cheng, S. Zhong, T. Xi, In vitro corrosion and biocompatibility of binary magnesium alloys, Biomaterials 30 (2009) 484–498.
[3] A.H. Martinez Sanchez, B.J.C. Luthringer, F. Feyerabend, R. Willumeit, Mg and Mg alloys: How comparable are in vitro and in vivo corrosion rates? A review, Acta Biomaterialia 13 (2015) 16–31.

11 Rheological Study of Core-shell Particles in Thermosensitive Copolymer Matrix Suitable for Drug Delivery Carriers

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The aim of this work was preparation and rheological study of thermosensitive polymer composite paste as a potential injectable composite material for drug delivery carriers. Newly prepared thermosensitive composite paste was consisted of aqueous solution of polymer matrix based on the hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(lactide—co—glycolide) (PLA/PGA) copolymer (PLGA—PEG—PLGA) thermogelling at body temperature with homogeneously distributed hydroxyapatite-polymer/core-shell (CS) particles1. The core in CS particles was composed from inorganic bioactive nano-sized hydroxyapatite (n-HAp) and covered by ITA/PLGA—PEG—PLGA/ITA copolymer shell. The polymer shell was additionaly end-crosslinked by carbodiimide coupling (zero-crosslinking). The original PLGA—PEG—PLGA copolymer matrix exhibited in aqueous solution two phase sol-gel and gel-suspension transitions. However, addition of 20 wt.% of CS particles into copolymer solution exhibited stable gel state without the sol-gel transition. However, composite containing up to 10 wt.% of CS particles increased the stiffness of the original hydrogel matrix almost 4 times (from 40 kPa up to 150 kPa) while maintaining two-phase sol-gel transitions.

References

[1] Vojtová L., Chamradová I., Michlovská L., Jančář J.: Czech Intelectual Property, Number 28 414 *„Thermogelling composite paste“*, 7. 7. 2015

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12 Multi-modal 3D Scanning for Medical Purposes

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3D printing and associated 3D scanning is rapidly spreading into the various domains related to human life. But still, there are mostly technical domains (at least a bit) getting involved. The medicine is one of domains, where 3D models are not much used yet, even though many opportunities of possible 3D scanning applications exist.

Next, there are various different imaging modalities and measuring systems used in present medicine, but all of them are used separately, without any interconnection.
Multi-modal data fusion of measured values and captured images, merging all heterogeneous data into one single multilayer 3D model could provide new information, which could help to find the right diagnosis and plan the appropriate therapy. Spatial resolution of such models can be also enlarged by one dimension and all these data could be registered in relation to time.

The core of proposed scanning and modelling system is formed by robotic 3D scanner [1], disposing with very high accuracy (up to 0.1 mm) and flexibility (due to use of 6 axis robotic manipulator) providing basic 3D model [2], onto which the data from other modalities are projected.

This work is focused on searching for medical 3D scanning applications, design of its technical solutions and development of appropriate hardware and software customized for specific medical applications.

References
[1] A. Chromy and L. Zalud, "Novel 3D Modelling System Capturing Objects with Sub-Millimetre Resolution," *Adv. Electr. Electron. Eng.,* vol. 12, no. 5, pp. 476–487, Dec. 2014.
[2] A. Chromy and L. Zalud, "Robotic 3D scanner as an alternative to standard modalities of medical imaging," *SpringerPlus*, vol. 3, no. 1, p. 13, 2014.

13 Assessment of cell properties of normal and transformed rat liver progenitor cells in 2D and 3D cultures

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WB-F344 is a rat liver epithelial cell line with characteristics of multipotent liver progenitor / oval cells, including expression of Oct-4, Sox-2, c-kit, or AFP. WB-F344 cells are characterized by functional gap-junctional intercellular communication (GJIC), contact inhibition of growth, lack of anchorage-independent growth (AIG), and ability of *in vivo* or *in vitro* differentiation into hepatic or biliary lineages. WB-F344 cells are non-tumorigenic, but can be neoplastically transformed in vitro by chemical treatment, oncogene overexpression or mutagenization. Transformed WB cells exhibit downregulated GJIC, reduced contact inhibition, increased AIG, and *in vivo* tumorigenicity.

In our study, we investigated differences between the cell phenotype and behavior of normal and transformed liver progenitor cells in 2D and 3D cell cultures. Real-time 2D impedimetric analyses (RTCA xCelligence) revealed that transformation of WB-F344 cells by different oncogenes (ras, myc, src, neu) induces characteristic changes of cell impedance and its time-profile, and indicated differences in cell adhesion, cohesion and contact inhibition between normal and transformed cell lines. WB cells were cultured also in 3D Petri Dish and evaluated by automated time-lapse microscopy, which showed altered spheroid formation and growth of transformed WB cells.

This in vitro system employing normal and transformed WB cells in combination with non-invasive label-free impedance- and imaging-based assays can be effectively used to study chemically- or oncogene-induced transformation of normal liver progenitor cells, or to study effects of chemopreventive and anticancer agents on transformed cells.

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14 The role of microRNA in cell cycle regulation and differentiation of human embryonic stem cell-derived neural stem cells

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Over the past decade, miRNAs were identified as crucial post-transcriptional regulators of gene expression. Recently, miRNAs were also directly linked with human embryonic stem cell (hESC) physiology and regulation of key stem cell properties: self-renewal and pluripotency. Importantly, we and others have previously connected self-renewal and differentiation with regulation of cell cycle. Here we aim to study the role of miRNAs in differentiation of hESCs into self-renewing neural stem cells (NSCs) and terminally differentiated neurons. We initiated our experiments by high throughput analysis (miRNAseq/RNAseq) of gene and miRNA expression in undifferentiated hESCs and their differentiated counterparts. Our results show that expression of several cell cycle regulatory molecules (such as p15/CDKN2B and p16/CDKN2A) remain low or undetectable in hESCs as well as self-renewing NSCs while they are markedly upregulated upon induction of terminal differentiation of NSCs into neurons. Furthermore, results from miRNA microarray analysis show 26 significantly upregulated miRNAs upon induction of differentiation of hESCs into NSCs. The most dramatically (>15 fold) upregulated miRNAs with differentiation were miR-21, miR-221, miR-125a, and miR-145. Some of these miRNAs are strongly associated with differentiation of hESCs and were shown to play a role in cell cycle regulation, i.e. miR-145 and let-7c. Other miRNAs have been strongly associated with various types of cancers, such as miR-21, miR-221/221 or miR-143. However, their role in differentiation of hESCs was never studied. Our investigations are therefore ongoing to reveal their target mRNAs and functions in cell cycle regulation and NSC phenotype. This work is supported by GJ15-18316Y.

15 Modular Dual-source tomographic scanner

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This work is focused on the description of recently developed modular multi - purpose X - ray device (patent pending EPO 14002662.6 - 1559) devoted particularly for computed tomography (CT) measurements [1]. CT scanner was designed as a complex and modular system with high flexibility The system arrangement is realized by two cross - oriented imaging lines X - ray tube – detector with shared rotational stage described in [2].

A major advantage compared to conventional CT systems is the ability of simultaneous measurement with both imaging lines. Both imaging lines can work at arbitrary magnification. There are two advantageous measurement arrangements.
- Dual Energy CT (DECT) for which data are recorded at two different energy spectra. Two image datasets are acquired and energy - dependent changes in the attenuation of different materials are analyzed.
- D ual Source CT (DSCT), which allows to obtain almost twice more projections with the same scanning time contrary to conventional tomography. Each imaging line works with the same energy spectra and acquires one half of the image dataset.

Technical solution of the system and respective results from computed tomography (CT) scanning using high resolution dual - source methods will be presented.

References
[1] Fila, T., Kumpova, I., Jandejsek, I., Kloiber, M., Turecek, D., Vavrik, D. Utilization of dual - source X - ray tomography for reduction of scanning time of wooden samples. (2015) *Journal of Instrumentation*, 10(05), pp. C05008 - C05008. DOI: 10.1088/1748 - 0221/10/05/C05008. ISSN: 1748 - 0221.
[2] Badea et al., Proceedings of SPIE, art. No. 691342.

16 Determination of Mean Diameters and Particle Size Distributions Using Analytical Centrifuge

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The characterization of the dispersed state of nanoparticles is essential. Techniques which avoid dilution, and therefore, do not modify dispersion properties are preferable. In this respect, although analytical centrifugation has significant potential, it has seldom been exploited. The measurement of the particle size distribution of dispersions by sedimentation in a centrifugal field is conducted by analyzing the variation of the extinction at one position over the measurement time using the line start or homogeneous technique. This method (Constant Position) leads to an integral which can only be solved approximately (all particles have to be homogenously distributed in the dispersion at the beginning). It is also necessary that all particles have passed the detector. A single measurement can take a considerable length of time, depending on the material properties of the dispersion. An alternative and faster solution is provided by analyzing the dispersion concentration over the entire sample length for a given time span. This method (Constant Time) has the advantage that an analytical solution with algebraic equations is possible, which enables a considerable reduction in the measurement time, since it is not a requirement that all particles are settled out [1].

In this work, we used different colloidal silica suspensions with defined mean diamters (50, 85, and 100 nm). Both analysis modes (Constant Position and Constant Time) provided the same results for individul samples. The mean diameters and particle size distributions are in very good agreement to reference silica particles. In this regard, the analytical centrifuge seems to be a very useful tool for particle size analysis.

References

[4] T. Detloff, T. Sobisch, and D. Lerche, Part. Part. Syst. Charact. 23 (2006) 184.

17 Automatic image analysis of 3D spheroids in toxicological research

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Formation and growth of cellular spheroids in scaffold-free systems have become one of the most commonly used 3D cell culture techniques. Spheroid cultures confer higher degree of biological relevance than traditional 2D monolayer cell cultures, and can be effectively used in toxicology to more reliably predict in vivo effects of toxic chemicals. The processes of spheroid formation and growth can be targeted and altered by toxicants, and their evaluation represents very sensitive and robust endpoint allowing quantification and characterization of chemical effects.

This poster will present our in-house developed ImageJ script allowing an easy and automated evaluation of spheroids. Script functions and performance were optimized and tested using spheroids formed in micromolded agarose hydrogels (3D Petri DishTM). Spheroids of several liver cell lines were evaluated, including normal and ras-transformed rat liver progenitor cells WB-F344, adult human liver stem cells HL1hT1, and human hepatocellular carcinoma cell line HepG2. We demonstrated that this ImageJ macro can effectively and automatically detect spheroids, quantify their numbers and also several parameters related to the spheroid size and shape (area, volume, perimeter, centroid, circularity, aspect ratio, roundness, solidity, Ferret diameter). In contrast to traditional methods based on manual analysis of microphotographs, this automated image analysis reduces significant bottleneck in the workflow of spheroid experiments, and allows rapid processing of digital microphotographs compatible with high-throughput toxicity testing.

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18 Current Transient in Metal–Semiconductor–Metal Structure of Schottky Contacts and Parameter Retrieval of CdTe Structures

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A new model to interpret the transient current in metal–semiconductor– metal (M-S-M) structure of two metallic Schottky contacts fabricated to low resistivity semiconductor material were proposed by considering the electromigration of ions in the depletion region formed at the reversed biased M-S interface. We assumed that the electric field confined in the depletion region causes electromigration at higher temperatures of the donor defects that are distributed in this region into the semiconductor bulk. The departures of these ions change with time the value of the electric filed, E_0, at the M-S interface. The field dependence of barrier height due to the image force was proposed to be the mechanism for the current through the structure, and therefore change with time of the current as a sequence of changing of E0 was proposed. The model was applied to the transient current measured on low-resistivity p-type CdTe with Au contacts and the diffusion coefficients of the donor ions were extracted.

19 Deciphering the puzzle of organelle-nucleous replicational couple using GRF5x*pfld Arabidopsis plants*

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Arabidopsis thaliana leaf development relies on subsequent phases of cell proliferation and cell expansion. During the proliferation phase, chloroplasts need to divide extensively, and during the transition from cell proliferation to expansion, they differentiate into photosynthetically active chloroplasts, providing the plant with energy. Over-expression of GROWTH REGULATING FACTOR 5 (GRF5-OE) enlarged leaf area (1) and increased the chloroplast number per cell (2). Evidence indicates that GRF5 and cytokinins synergistically enhance cell division and chloroplast division. Moreover, a possible function for the chloroplast tetrapyrrole retrograde signal to coordinate chloroplast division and cell division via GRF5 induction of chlorophyll biosynthesis genes expression was proposed (2). Analysis from transgenic plants expressing a cyanobacterial flavodoxin in chloroplast (*pfld*, 3) revealed a specific up-regulation of genes related to chlorophyll biosynthesis and involved in retrograde signaling.

In order to decipher the puzzle of organelle-nucleus replicational couple and shed light on largely unknown regulatory circuits engaged between organelle and nucleus to synchronize chloroplast and cell division (4), crosses between GFR5-OE and *pfld* plants were generated. Results will provide better understanding about how chloroplast and nuclear signals are integrated to regulate leaf growth and development.

References
1. Gonzales et al. (2010). Plant Physiology.
2. Vercruyssen et al., (2015). Plant physiology.
3. Zurbriggen et al. (2008). Trends Biotechnol. 26: 531-537.
4. Rintamäki et al. (2009). Plant Signaling and Behaviour.
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20 Comparison of cell colonies and singel cells using image analysis methods and high content screening

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High content screening microscopy systems produce increasing amount of image data and this trend goes along with grow of computational power. The strength of results and conclusions obtained by such experiments is inevitably bigger. On the other hand manual methods used for decades for cell cytometry, cell and colony counting are not possible to use anymore, mainly because manpower needed for the evaluation of thousands of images would be immense. Therefore rising demand for automatic evaluation methods in image cytometry is also unexceptionable.

Here we reveal workflow of image capturing, processing and analysis combined with stereology methods used for comparison of colony size between two subpopulations of differently expressing TROP-2 molecule in mouse mammary carcinoma 4T1 cells and human prostate cancer cell line derived from brain metastasis DU145. The power of automated methods is even more obvious on a single cell level where we compared DU145, docetaxel-resistant DU145 and matching non-resistant control cells

Furthermore, for the image evaluation we used freeware tools - CellProfiler, Fiji and Ilastik. Therefore presented workflow could be adopted by other groups dealing with large data sets captured on microscopes.

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21 Eye and lens development studied with microCT

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With lab-based microCT it is possible to examine small organs, such as the eye and lens of a mouse embryo with 3D histological detail. The objective of this project is to visualize the organogenesis of the mammalian eye with a specific focus on the differentiation of the ocular lens.

Cataracts, an opacity of the lens, often appear with increasing age, but also can be congenital (even inherited), and in children this condition demands immediate surgery. Cataracts are known to be a result of mutations in regulatory genes (e.g. *Pax6*) or in structural proteins (e.g. MIP) that influence the morphogenesis of the lens and the eye itself. The medical motivation for further research on this topic is evident: micro CT imaging could help to visualize the development of a wild type (or normal) eye, and the development of the eyes of known mutants to better understand the origins and consequences of the disease. Like humans, mice have a typical mammalian eye, and are thus a suitable model for both species.

High-resolution microCT images were made of phosphotungstic acid (PTA) stained mouse embryos (8.0 dpc to 9.75 dpc) with an Xradia MicroXCT system. Because the lens is avascular and lacks any innervation, it should be possible to visualize the cells of both lens epithelium and lens fibres to create an accurate image of the structure at critical stages of development. Preliminary images show precise demarcation between the lens and the surrounding layers, demonstrating that formaldehyde fixation and PTA staining are sufficient to give an overall image of the eye with sufficient resolution to investigate details of the developing eye and lens.

22 A novel PLLA/nacre and pearl powder scaffold for bone repair

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In order to fabricate an ideal scaffold for bone repair, PLLA, PLLA/aragonite pearl powder and PLLA/vaterite pearl powder scaffolds were fabricated by freeze-drying[1]. Composite scaffolds and PLLA scaffold displayed porous structures. The addition of pearl powder increased the compressive strength and the compressive modulus of the scaffolds. The hydrophilicity of composite film increased in the following order: PLLA/aragonite > PLLA/nacre > PLLA/vaterite, which was in accord with the result of protein adsorption.

In vitro cell culture showed that PLLA/aragonite and PLLA/nacre stimulated proliferation and osteogenic differentiation of rat bone mesenchymal stem cells (rBMSCs). The in vitro degradation showed that both composite scaffolds had a similar degradation behavior. During the degradation, with the dissolution of pearl powders, the composite scaffolds displayed a gradual decrease of bulk density and mechanical property, which were still higher than that of the PLLA scaffold.

Bone regeneration was evaluated using a rabbit radial defect model. The results suggested a significant increase in bone formation in PLLA/aragonite scaffold at 8 weeks compared with other groups, and the newly formed bone was similar to natural bone at 12 weeks. PLLA/vaterite scaffolds enhanced bone formation at 12 weeks. All results revealed that PLLA/aragonite and PLLA/vaterite scaffolds significantly increased bone regeneration, which provided theoretical and practical supports for their clinical applications.

References

[1] Liu Y S, Huang Q L, Feng Q L. 2013. Biom. Mater.8,065001-065010.

23 3D modeling of hierarchical biocomposites

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Biological structural materials receive increasing attention by material scientists because they have been optimized during evolution and they are therefore ideally suited to study the efficiency of nature's design principles. These materials differ fundamentally from most man-made structural materials in being structurally heterogeneous by combining different in/organic constituents into composites with hierarchical organization. We propose a hierarchical model for the prediction of the elastic properties of a mineralized arthropod cuticle using quantum-mechanical calculations to find the elastic properties at the nanoscale and employing hierarchical homogenization to find the cuticle properties at all hierarchy levels. Based on our results we suggest that the mineral-protein matrix possesses a microstructure (so-called symmetric cell material) which exhibits extremal properties in terms of stiffness. We also discuss the role of chitin and the multifunctional optimization of the cuticle in terms of a trade off between stiffness and transport capacity of the pore canal system [1-3]. Recently, we have further extended our study to analyze the stiffening impact of magnesium additions on Mg-containing calcite particles [4].

References

[5] S. Nikolov *et al.,* Advanced Materials 22 (2010) 519.
[6] S. Nikolov *et al.,* Journal of the Mech. Behav. of Biomed. Mater. 4 (2011) 129.
[7] M. Petrov *et al.,* Biopolymers 99 (2013) 22.
[8] L.-F. Zhu *et al.,* Journal of the Mech. Behav. of Biomed. Mater. 20 (2013) 296.

24 Calcium and Calcineurin-NFAT Signaling Regulate Granulocyte-Monocyte Progenitor Cell Cycle via Flt3-L

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Maintenance of myeloid progenitor cells is controlled by complex regulatory mechanisms and is orchestrated by multiple different transcription factors. Here, we report that the activation of the transcription factor nuclear factor of activated T cells (NFAT) by calcium-sensing protein calcineurin inhibits the proliferation of myeloid granulocyte–monocyte progenitors (GMPs). Myeloid progenitor subtypes exhibit variable sensitivity to induced calcium entry and consequently display differential engagement of the calcineurin-NFAT pathway. This study shows that inhibition of the calcineurin-NFAT pathway enhances the proliferation of GMPs both in vitro and in vivo and demonstrates that calcineurin-NFAT signaling in GMPs is initiated by Flt3-L. Inhibition of the calcineurin-NFAT pathway modified expression of the cell cycle regulation genes Cdk4, Cdk6, and Cdkn1a (p21), thus enabling rapid cell cycle progression specifically in GMPs.

NFAT inhibitor drugs are extensively used in the clinic to restrict the pathological activation of lymphoid cells, and our data reveal for the first time that these therapies also exert potent effects on maintenance of the myeloid cell compartment through specific regulation of GMP proliferation.

Cell to cell interactions in the bone marrow stem cell niche provide fundamental stimulation regulating early hematopoiesis. We plan to use 3D co-culture of human hematopoietic stem and progenitor cells with mesenchymal stem cells to mimic cell signaling within the stem cell bone marrow niche. This experimental tool will allow validating our findings in patients' derived hematopoietic cells and screening the candidate molecules important in hematopoiesis regulation.

Reference

[9] Fric J et al. Calcium and calcineurin-NFAT signaling regulate granulocyte-monocyte progenitor cell cycle via Flt3-L. Stem Cells 32, 3232-3244 (2014)

25 Immunomodulation of the endogenous cytokinin content as the new tool for discovery of cytokinin subcellular-specific functions

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Cytokinins represent a group of plant hormones of different chemical composition. Cytokinin receptors were found to localize to endoplasmic reticulum (ER) and recognize individual cytokinin types with different affinity. However, our knowledge on specific role of individual cytokinin types and their metabolites as well as the functional importance of ER-located cytokinin receptors is scarce. We manipulated cellular distribution of cytokinin trans-zeatin riboside (tZR) in Nicotiana tabaccum stable transgenic lines via overexpression of recombinant tZR specific single-chain variable fragments (αtZR_scFvs) targeted to ER. The overexpression of αtZR_scFvs results into strong downregulation of tZR but not of the free base trans-zeatin. The misregulation of cytokinin ribosides results into changes of the entire cytokinin metabolism. Under normal conditions, we were unable to identify any obvious phenotypic aberrations in the 35S:αtZR_scFvs plants. However, the transgenic lines reveal hypersensitive response to exogenously applied cytokinins. Using TCS:LUC reporter in tobacco protoplasts we showed the ability of ER-targeted αtZR_scFv downregulate cytokinin signaling. It suggests that the ER localized cytokinins could be functionally important and that the signaling could be triggered from ER compartment through the cytokinin receptors localized in the ER membrane. FLIM/FRET analysis revealed interactions between ER-localized cytokinin receptors and their downstream signaling targets, the histidine-containing phosphotransfer proteins, clarifying thus the link between ER-localized cytokinins and downstream cytokinin signal transduction. Altogether, our data provide the evidence for the functional importance of ER-located cytokinin ribosides and cytokinin receptors in plants.

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26 Shaping of bioceramics on micro level via dip-coating process

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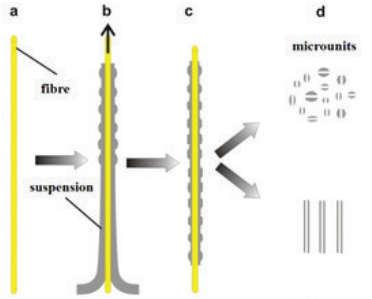
Nowadays, several methods are used for shaping of 3D bioceramic scaffolds, however production of scaffolds, for example for nutrition delivery, is difficult and expensive. In this study, we describe an easy and inexpensive method to create 3D hydroxyapatite structure containing porous hollow fibers and beads via dip-coating. This method use micro fibers coated by hydroxyapatite suspension, which are sintered to achieve micro-channels and replicate shape of the fiber. The proposed straightforward method for building 3D bioceramic structures is suitable for in vitro bioreactors studies as well as for hard tissue engineering.



a) fiber before coating
b) coating the suspension to the fiber
c) green body
d) beads and hollow fibers after sintering

27 Macroporous hydrogels prepared by the electron-beam initiated cryopolymerization: the tailoring of the porosity and elasticity

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Fast developing of tissue engineering and cell cultivation fields of industry requires new materials with desired set of features such as suitable for the cell growth architecture, mechanical properties and surface composition. Representing a group of macroporous hydrogels with interconnected pores system [1] cryogels show spongy structure with high swelling capacity while preserving good mechanical stability. In the present work, a technique for the modification of the pore size and stiffness of acrylamide-based cryogels is demonstrated via the regulation of an electron beam irradiation dose. Samples were characterized by equilibrium swelling measurements, light and scanning electron microscopy and stiffness measurements. A ^{125}Iradiolabeled azidopentanoyl-GGGRGDSGGGY-NH_2 peptide was bound to the surface to determine the concentration of the adhesive sites able for biomimetic modification. The functionality of the prepared substrates was evaluated by in vitro cultivation of adipose-derived stem cells. Moreover, the feasibility of preparing layered cryogels was demonstrated. This may be the key to the future preparation of complex hydrogel-based scaffolds for a wide range of applications.

References

[10] 1. Lozinsky, V. I. et al., Trends Biotechnol. 21, 445–451 (2003)

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28 Myelopoiesis under HIF-1 modulation

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Myeloid cells (granulocytes and monocytes) are derived from multipotent hematopoietic stem cells and finally are key effectors of the innate immunity. Hypoxia and hypoxia inducible factors (HIFs) play an important role not only for migration and cell survival but also as a modulation signal for myeloid progenitors in hematopoietic organs.
Here we focus on the role of HIF family member HIF-1α in regulation of myelopoiesis, one branch of hematopoietic differentiation.

It has been observed that deferoxamine (DFO) and other Fe ions chelators not only induce differentiation but also shift myeloid differentiation to monocyte lineage in acute myeloid leukemia (Callens et al., 2010). DFO through Fe ion chelation also stabilizes HIF-1. Therefore we tested the effect of classical HIF stabilizing drugs ($CoCl_2$ and DMOG) on adult and fetal hematopoiesis.

Interestingly, $CoCl_2$ decreased CFU-G together with CFU-GM but increased CFU-M number in fetal haematopoiesis in the same manner as DFO in acute myeloid leukaemia study (Callens et al., 2010). In contrast, in adult hematopoietic progenitors $CoCl_2$ increased number of CFU-G and CFU-GM but decreased CFU-M.

In summary, our results show HIF-1α dependent regulation of myeloid lineage directed differentiation. Moreover, the results of myeloid differentiation in response to $CoCl_2$ in adult and fetal hematopoiesis may indirectly support recent hypothesis, that tumor cells may have the phenotype of fetal progenitors rather than the phenotype of adult lineage progenitors.

References
Callens C et al. (2010) *J Exp Med.* 207(4):731-50

29 Application of mass spectrometry in determination of enzyme inhibition and inhibitor screening

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Enzyme kinetics plays an essential role in the regulation of all processes of life. Furthermore, enzymes are an important class of drug targets for the treatment of several diseases. Screening for inhibitors of pharmacologically-relevant enzymes is in many cases an important starting point in drug discovery [1,2]. In recent years, mass spectrometry (MS)–based assays have been applied as alternative detection methods for high-throughput screening (HTS). MS offers the remarkable advantage that it does not require any analytes to be labelled, either by direct attachment of fluorescent and radioactive labels or by binding of antibodies, and therefore offers better flexibility in research field [3]. In this paper, APCI-MS-based assay has been developed to determine urease activity and inhibitor screening, including natural products extracts. In addition, we present the recent advances in the development of MS-based detection procedures capable of assaying enzyme inhibition and inhibitor screening.

References

[11] R.A. Copeland. Evaluation of Enzyme Inhibitors in Drug Discovery: A Guide for Medicinal Chemists and Pharmacologists, Wiley Interscience: Hoboken, NJ, 2005; p 296.
[12] O. von Ahsen, U. Bomer. High-Throughput Screening for Kinase Inhibitors. ChemBioChem. 2005; 6: 481–490.
[3] J. Wu, S. Takayama, CH. Wong, G. Siuzdak. Quantitative electrospray mass spectrometry for the rapid assay of enzyme inhibitors. Chem Biol. 1997; 4: 653-657.

30 A new integrated solution for volume reconstruction of biological samples

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The Teneo VS SEM represents a new integrated solution for SEM (scanning electron microscopy) volume data acquisition based on a refined SBFSEM (Serial Block-Face SEM) technique [1]. The Teneo SEM, in addition to a newly developed in-situ microtome and the reconstruction software form the key components of the workflow used for automatic data acquisition from selected regions of interest in resin embedded samples stained with heavy metals. The volume information is revealed by physical and/or virtual slicing depending on the required depth resolution and sample conditions. The pure SBFSEM mode is based on alternate slicing and backscatter imaging of the block-face. For virtual slicing a series of images of the exposed block-face is acquired using different accelerating voltages. By their proper selection different backscatter depth emission profiles are created. The collected images may serve as the input for a deconvolution algorithm that computes several subsurface layers. This approach is based on the multi-energy deconvolution SEM technique capable of high-resolution reconstruction of the top layers of the sample [2].

References
[1] B. Titze et al., Journal of Microscopy, vol. 250(2) (2013).
[2] F. Boughorbel et al., patent US 8,232,523 B2 (2012).

31 BIOLOGICAL PROPERTIES OF VARIOUS POLYANILINE FORMS

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Polyaniline is a promising conducting polymer which can be preferentially applied in tissue engineering of hearth and nervous tissues. Despite of this potential, its biological properties has been studied to less extent, compared with other conducting polymers. The significant improvement of knowledge was reached thanks to the cooperation of authors of this abstract and their co-workers. As a polyaniline can be in a form of powders, films or colloids, mentioned team has focused on all of them. The polyaniline powders were studied mainly in terms of their cytotoxicity as the monomer and reaction intermediates are known to be harmful in terms of carcinogenity and toxicity. Firstly the cytotoxicity of polyaniline powders prepared according to the IUPAC were therefore detected (1) and subsequently its purification were outlined (2,3). Antibacterial activity, cytotoxicity and neutrophil activation of colloidal dispersion (4) were studied for the first time to reveal its applicability. Finally, the interaction of polyaniline films with eukaryotic cells (5) were studied with the aim to use those knowledge for preparation of 3D structures combining the biopolymers and conducting polymers into the scaffolds for tissue engineering.

References

[13] Humpolíček P., et al. Synthetic Metals. (2012).
[14] Stejskal J., et al. Synthetic Metals. (2014).
[15] Kašpárková V., et al. Polymers for advanced technologies (2015).
[16] Kuceková Z., et al. Colloids and Surfaces B: Biointerfaces (2014).
[17] Bober P., et al. RSC Advances. (2015).

32 Correction of the focal spot drifting in X-ray μ-tomography

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Nowadays micro-focus X-ray tubes in conjunction with digital pixelated imagers are standardly utilized for micro-radiography; radiogram resolution can be even better than one micrometer with high projection magnification. The same requirement for computed tomography (CT) is not easy to fulfill due to time-dependent focal spot position drifting and tube thermal deformations. These effects can be significant especially for long-time measurement, which is necessary for high quality micro-CT. In this work, a method for correction of this effect is proposed. The method is based on the regular recording of an object zero position throughout the tomographic sequence. A shift of the object between those images caused by the focal spot drifting is measured employing an image correlation technique. Obtained knowledge of the shift is included into calculations of the final reconstruction. A distinct improvement in reconstruction results is shown in the case of plastic composites.

33 Microfluidic systems for establishment of stem cell niche

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Microfluidic systems offer the possibility to create microenvironment for applications of stem cell expansion and differentiation. Cellular behavior is influenced by chemical and physical signaling in their niche which can be established by structural design of microfluidic device, flow rates and properties of surrounding gels.

The major advantage of these systems is the nutrient/waste exchange via microperfusion. It allows spatial and temporal control of transport of fluids and soluble factors (e.g. growth factors, chemokines and hormones), as well as creating gradient concentration of biochemical signals.

In this work, we are presenting several systems as effective research tools for creating stem cell niche and studying of cellular behavior.

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34 Structural study of ETR1 using protein trans- splicing

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Ethylene was the first gaseous biological signalling molecule discovered. As a plant growth regulator factor, it is involved in several developmental processes like seed germination, seedling growth, leaf, root, stem and flower development, fruit ripening, senescence, and responses to variety of stresses. Perception of ethylene and the signal transduction into the cell is achieved by membrane receptors, to which ethylene receptor 1 (ETR1) belongs. ETR1 is a multi-domain protein which contains a hydrophobic N- terminal trans-domain that encompasses the ethylene-binding site, followed by a large cytosolic domain consisting of GAF domain, transmitter histidine kinase and response regulator (or signal receiver). We successfully expressed the receiver domain of ETR1, which monomeric form resembles the bacterial receiver domain. Although the crystal structure of ETR1RD has been already determined, its intermolecular interactions and the role of divalent ions are experimentally not well defined. For this purpose, we designed an ETR1RD construct with an intein. The intein sequence has been added to the N-terminus of the receiver domain and expressed in ER2566 E. coli host strain. By using protein trans-splicing as a post-translational modification, we ligated two flanking N- and C-terminal segments (N- extein and C- extein) by a peptide bond and concomitantly cut the sequence of interest out from the precursor protein and achieved to obtain a ligated soluble protein. The results showed that the protein trans-splicing was successful and can be used for the upcoming segmental isotopic labeling of our multi-domain protein.

35 Plasmochemical synthesis of low dimensional carbon nanomaterials in a dielectric barrier discharge

Petr Jelinek

The atmospheric pressure dielectric barrier discharges (DBD) are sources of non-equilibrium plasma in which at least one dielectric layer is placed between the electrodes [1]. Dielectric barrier discharges were originally used mainly for ozone production [2] because their tendency to form filaments did not permit applications in the field of material science. In last two decades were however developed many experimental approaches (based on work of Okazaki et al. [3]) which allows set up experimental condition in way which suppressed the formation of filaments in the discharge.

The aim of this work was to synthesis low dimensional carbon nanomaterials (especially graphene and multi-layer graphene) in an atmospheric pressure dielectric barrier discharge (DBD). Nearly homogeneous regime of the DBD was used during the synthesis and precursor (methane CH_4 or acetylene C_2H_2) were directly mixed in the deposition reactor with argon. The depositions were carried out in an atmospheric pressure DBD ignited by a sinusoidal high voltage (7.1 kHz) supplied with a tunable generator providing 12 W power input. Pieces of silicon wafer (10x15 mm) with 100 nm thermal silicon oxide overlayer with thermally evaporated copper in form of 8 mm diameter disc (thickness 300 nm and 500 nm) were used as substrate during synthesis because of low solubility of carbon in copper in temperature bellow 1000°C. The substrate holder was heated by external heater to temperature from 650°C to 800°C.

Multilayer graphene structure was deposited using experimental condition described in previous part. Beside of the graphene it is possible prepare other carbon materials (nanotubes) just by adjusting experimental parameters and by choosing right type of substrate. Deposited materials were evaluated by Raman spectroscopy and SEM.

Using DBDs for the graphene synthesis have many advantages in comparison with production by chemical vapour deposition, because temperature of substrate can be, because of decomposition of precursor in the discharge, significantly lower and functionalization of deposited layer can be done in situ, which also lower number of the contaminants on the layer.

36 An insight into the role of HMGB proteins in human pluripotent and multipotent cells

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High mobility group proteins (HMGBs), which are known as architectural non-histone chromosomal proteins, are involved in various processes including maintenance of genomic integrity of eukaryotic cells. Dysregulation of HMGB proteins and its effects have been described in many types of cancers, however, relevant information for noncancerous pluripotent cells is not available. Here we have studied the effects of down-regulation of HMGB1, HMGB2, and their combination in undifferentiated pluripotent human embryonic stem cell (hESCs) and multipotent human neuroectodermal cells (hNLCs). To do so, we have established a series of stable lines of hESCs expressing shRNAs against the given HMGBs under the influence of doxycycline. The major phenotypes that we have observed were as follows. Down-regulation of HMGB2 and also HMGB1+HMGB2 caused increase in proliferation speed of hESCs but this was not the case in hESCs with down-regulated HMGB1. The same pro-proliferative effect of down-regulation of HMGB2 was observed also in hNLCs. In contrast to the effect of down-regulation of HMGB2, down-regulation of HMGB1 in hNLCs resulted in induction of apoptosis, as evidenced by increased levels of cleaved PARP and caspase-3, and also p53 and other cell death-associated molecules. Interestingly, no such proapoptotic effect was observed in undifferentiated hESCs. Similarly, when hESCs and hNLCs with down-regulated HMGBs and their combinations were exposed to DNA-damaging agent etoposide, only hNLCs with down-regulated HMGB1 showed signs of apoptosis. All other "mutants" remained unaffected. Finally, down-regulation of HMGBs affected the process of differentiation of hESCs towards neural lineage. Specifically, down-regulation of HMGB1 significantly decreased the number of neural rosettes, while down-regulation of HMGB2 their number slightly increased. Together, although it is not yet fully conclusive, it seems that in hESCs and also hNLCs the HMGB1 and HMGB2 proteins affect some fundamental processes in an opposite manner.

37 Optimal Conditions for Opening of Membrane Pore by Amphiphilic Peptides

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Amphiphilic peptides can interact with biological membranes and severely affect their barrier and signaling functions. These peptides, including antimicrobial peptides, can self-assemble into transmembrane pores that cause cell death. They are important part of the natural defense system of many organisms. Despite their medical importance, the exact mechanism of action and the conditions needed for pore formation remain elusive.

Using Monte Carlo simulations and coarse-grained models, we have systematically investigated the free energies associated with peptide pore formation under various scenarios; including different peptide concentrations, lengths, and arrangements. In agreement with experiment, we found that high peptide-to-lipid ratio is necessary for spontaneous pore assembly – increasing peptide concentration decreases the barrier for pore opening. The peptide length has a non-monotonic impact on pore formation and the optimal length matches the membrane thickness. Furthermore hydrophobicity of peptide ends and mutual positioning of peptides on the membrane play a role.

38 Biological properties of chitosan/collagen/hyaluronic acid composites

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Collagen and chitosan are widely used as biomaterials. Hyaluronic acid is a polysaccharide which is biocompatible, biodegradable and non-toxic for human body. Such biopolymers can be used in tissue engineering science where scaffolds made of them are useful for the new living tissue growth.
Collagen and chitosan were mixed in weight ratio 50/50. Addition of 1, 2 and 5% of hyaluronic acid was used and 3D porous scaffolds were obtained due to the lyophilization process. Scaffolds were sterilized by immersion in 70% ethanol and washed by PBS (pH=7.4). Then they were stored in DMEM medium for 24h. Cells were seeded on the scaffolds in density 200 000 SaOS cells per 150 µl of PBS. After 1, 7 and 14 days the cells were extracted with 1% Triton-X for 1h at 4oC. The extracts were frozen at -20oC and the alkaline phosphatase activity assay analysis was made.

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References
[18] A. Sionkowska, B. Kaczmarek, K. Lewandowska. Modification of collagen and chitosan mixtures by the addition of tannic acid, Journal of Molecular Liquids, 199 (2014) 318-323.
[19] A. Sionkowska, B. Kaczmarek, J. Stalinska, A.M. Osyczka. Biological properties of chitosan/collagen composites, Key Engineering Materials, 587 (2014) 205-210.
[20] A. Sionkowska, J. Kozlowska. Properties and modification of porous 3-D collagen/hydroxyapatite composites, International Journal of Biological Macromolecules, 52 (2013) 250-259.

39 Recreating a bioartificial vascularized microenvironment for pancreatic islet transplantation

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One of the effective physiological corrections of the insulin-dependent diabetes is the replacement of pancreatic islets. The current Edmonton protocol of the intra- hepatic portal islet transplantation has limited clinical success due to the progressive loss of graft viability and function, caused majorly by the poor revascularization of graft besides several other transplantation site associated factors. Therefore, the extra-hepatic islet transplantation methods are in high demand to provide a viable long-term answer for the patients suffering from the insulin-dependent diabetes. To this end, here we hypothesize to create a subcutaneous, highly vascularized, bioartificial microenvironment that meets the native physiological oxygen demands of pancreatic islets. The current study, the first of its kind, describes the use of polylactide-based, capsule shaped, anisotropic channeled porous scaffolds, functionalized with vascular endothelial growth factor, for the bioengineering of a subcutaneous vascularized chamber using the host body as a living bioreactor. The in vivo studies in rats, supported by experimental MR imaging, histochemical and immuno-histochemical evaluations, were promising. Studies are in progress to transplant the islets in these pre-vascularized chambers and to track the diabetes reversal.

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40 Biocompatible polymeric nanofibers modified with silk sericin

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Silk sericin is bioactive molecule isolated from silkworm cocoons. Sericin has been previously shown to have positive effect on cell proliferation and to also pronounce antioxidative and antimicrobial properties[1]. Therefore it may hold a promise as an additive to polymers to be used as cell carriers for regenerative medicine.

Here we have prepared polymeric nanomaterials from biocompatible synthetic polymers that have been modified by various concentrations of silk sericin in order to improve bioactive properties of such cell supports. Nanomaterials were prepared by electrospinning method using Nanospider™ technology. These nanomaterials were then evaluated for their physicochemical properties as well as for their influence on cell behaviors in *in vitro* cultures. Specifically, we have used light and scanning electron microscopy to unravel the detailed morphology of the interaction between materials and cells. The cell types used here were as follows: human lung carcinoma cells – line H441, normal human mesenchymal stem cells isolated from lipoaspirate, and normal neural progenitors differentiated in vitro from human embryonic stem cells.

Most importantly, we show that addition of silk sericin promotes growth of model cells used here and also positively influences intimacy of the interaction of these cells with nanofibrous materials. These findings open new area for development of biocompatible and bioactive cell supports for tissue engineering applications.

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1. Sehnal F. Prospects of the practical use of silk sericins. Entomol Res. 2008;38:S1-S8. doi:10.1111/j.1748-5967.2008.00168.x.

41 Function of cyclin-dependent kinase 12 during mouse development

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Gene expression is a tightly controlled process involved in a broad spectrum of biological processes, ultimately giving cells the ability to take control of their growth, apoptosis, differentiation and developmental potential. Regulation of gene expression is orchestrated primarily at the level of transcription of specific mRNAs by RNA polymerase II (RNAPII) and its activity is primarily regulated at the level of posttranslational modifications; among them, phosphorylation is the most prevalent. We have demonstrated that the cyclin-dependent kinase 12 (CDK12) phosphorylates RNAPII and thereby regulates expression of several different sets of genes, such as DNA-damage repair, lipid metabolism and nucleic acids synthesis. Yet, the function of CDK12 in development has not been demonstrated as of today. Thus, we decided to examine the role of CDK12 in mouse development. First, we employed TALEN (transcription activator-like effector nucleases) technology to generate a complete knock-out mouse with deletion of several nucleotides in exon 4 of CDK12 gene leading to expression of truncated CDK12 form in all cells. Mice with wild type allele were born at regular rates and appear normal; however, CDK12 complete knock-out mice were not born at all. Detailed analyses revealed lethality of complete knock-out mice around 5.5 days post conception resembling severe developmental defects. At the moment, we are dissecting functional consequences of CDK12 loss-of-function during the preimplantational stage.

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42 3D models of mammary branching morphogenesis reveal a pleiotropic role for FGF signaling in mammary stroma

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FGF signaling is a crucial pathway that regulates mammalian development from early embryonic stages throughout lifetime. Among other functions, FGF signaling mediates epithelial-stromal interactions necessary for proper tissue development and homeostasis. In mammary gland, FGF signaling has been demonstrated to regulate epithelial branching and mammary stem cells; however, its role in mammary stroma has not been elucidated. Our study shows that FGF signaling is functional in primary mammary fibroblasts and is mediated by FGFR1c, which activates downstream MAPK signaling cascade and expression of FGF target genes. FGF2 treatment, and to a lesser extent FGF9 treatment, promotes fibroblast migration, proliferation and extracellular matrix (ECM) remodeling. Moreover, using a 3D spheroid invasion assay we show that FGF2 induces an invasive phenotype in fibroblasts through its action on actomyosin cytoskeleton. Inhibition of MLCK or small GTPases Rac1 and Cdc42 abrogates FGF2-induced invasion, while inhibition of ROCK strongly induces the invasive phenotype. 3D organotypic co-cultures of mammary epithelial organoids with fibroblasts revealed that this invasive phenotype is induced by mammary epithelium and represents an activated state of fibroblasts that regulates mammary branching morphogenesis through both paracrine signaling and ECM remodeling.

Overall, our findings suggest a pleiotropic role for FGF signaling in mammary fibroblasts and bring new insights into mechanisms that regulate mammary gland development and homeostasis, and deregulation of which could lead to tumor formation.

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43 PROTEOMIC CORE FACILITY AT CEITEC-MU

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The Masaryk University CEITEC Proteomic Core Facility responds promptly to groving level of concern over needs for advanced proteomic technologies. The CF provides academic community and other subjects with access to high-end proteomic instrumentation based on shared resources and highly trained staff. The expensive instrumentation and specialized know-how enable the CF to meet demands of the research community and contribute to the effective utilization of resources as well. Projects mostly involve collaboration between multiple groups with special expertise. The Proteomic CF staff is open to increasing demand for intellectual involvement and researches are encouraged to approach the facility for expert advice in terms of discussing the merits/flaws of proteomics for their research, planning experiments, budgeting costs, possible outcomes and technical details.

The Core Facility is part of Czech National Affiliated Centre of INSTRUCT and participates in the Association of Biomolecular Resource Facilities ABRF. Czech and international researchers from universities and research institutes interested in accessing the core facility can benefit from support of CEITEC – open access project funded by the Ministry of Education, Youth and Sports of the Czech Republic.

44 CELLULAR UPTAKE OF UPCONVERSION LANTHANIDE NANOPARTICLES CONJUGATED WITH TAT- AND RGD-PEPTIDES

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Upconversion nanoparticles are unique luminescent materials which can be excited with NIR to emit visible light via an energy transfer upconversion mechanism [1]. $NaYF_4:Yb^{3+}/Er^{3+}$ nanocrystals are example of most efficient upconversion phosphors (Figure 1 a). Reverse microemulsion technique was selected as a suitable method for fabrication of silica shell around the $NaYF_4:Yb^{3+}/Er^{3+}$ nanocrystals (Figure 1 b).

Confocal inverted fluorescent microscopy was used to examine internalization of the $NaYF_4:Yb^{3+}/Er^{3+}$ crystals and their modification with TAT and RGD peptides in the human cervix carcinoma (Hela) cells (Figure 1 c). Easy monitoring of the TAT- and RGD-conjugated $NaYF_4:Yb^{3+}/Er^{3+}$ nanocrystals in living cells and perspective attachment of drugs makes such nanocrystals promising tools in theranostic applications.

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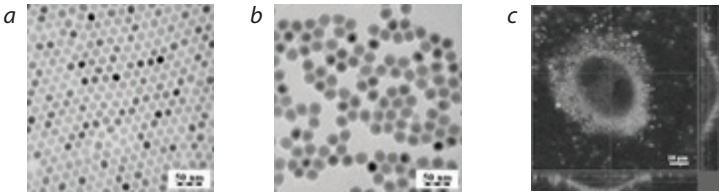

Figure 1. TEM micrographs of (a) NaYF4:Yb3+/Er3+ and (b) $NaYF_4:Yb^{3+}/Er^{3+}\&SiO_2$ nanoparticles. (c) Luminescence image of HeLa cell after incubation with $NaYF_4:Yb^{3+}/Er^{3+}\&SiO_2$-TAT nanoparticles.

[21] Auzel F., Upconversion and anti-Stokes processes with f and d ions in solids, Chem. Rev. 104, 139–173 (2004).

45 Collagen/chitosan porous scaffolds with improved biostability for tissue engineering

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Collagen is readily available and it possesses many interesting properties, such as biocompatibility, non-antigenecity, non-toxicity. For this reason, this protein is regarded as one of the most important and useful biopolymer in biomaterial's research [1]. Collagen-based materials are widely used in tissue engineering. However, the disadvantage of using collagen as a biomaterial for tissue repair is its high degradation rate, which leads rapidly to a loss of mechanical properties [2] Many attempts have been made to overcome this problem through the means of mixing collagen with either natural or synthetic polymers or different crosslinking method [3].

In this work a porous collagen/chitosan/poly(ethylene)glycol matrices were fabricated by the *freeze-drying* method. In order to improve especially the mechanical properties and *susceptibility* to *degradation* of the materials, the scaffolds were physically modified using a *dehydrothermal treatment* (DHT). The aim of present study was to investigate the influence of addition a small amount of poly(ethylene)glycol on the properties of collagen/chitosan scaffolds. The effects of poly(ethylene)glycol addition and crosslinking were examined using measurements: water uptake ability, enzymatic degradation, porosity and mechanical properties.

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References
[22] C.H. Lee et al. Int. J. Pharm, 221 (2001) 1-22.
[23] X. Liu et al. Biomed. Eng. 32 (2004) 477-486.
[24] R. Parenteau-Bareil et al. Materials 3 (2010) 1863-1887.

46 TUSC3 loss enhnaces ovarian cancer malignancy by promoting the ER stress response

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Ovarian cancer (OC) is one of the most serious cancers in women and despite recent progress in diagnosis and therapy, the prognosis for patients with OC is unfavorable, mostly because of late diagnosis and lack of reliable prognostic and predictive biomarkers. Recently, we described that epigenetic silencing of TUSC3 correlates with poor overall and disease-free survival of OC patients. TUSC3 is a putative tumor suppressor located in the membrane of endoplasmic reticulum (ER) participating in protein N-glycosylation. It is well established, that disturbances in N-glycosylation are associated with cancer development, however, the precise molecular mechanism, including the role of TUSC3, remains unclear.

In our model, TUSC3 loss enhanced growth of OC cells in mice confirming the status of TUSC3 as a tumor suppressor, moreover, it altered ER stress response and promoted epithelial-to-mesenchymal transition in OC cells. In addition, TUSC3 downregulation resulted in earlier formation of 3D spheroids under low-adhesion condition and provided resistance to N-glycosylation inhibition-induced decomposition of spheroids. We conclude, that the cumulative effect of TUSC3 silencing and extrinsic signals triggering the ER stress response contributes to EMT and tumor dissemination as well as tumor progression in OC.

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47 Post-translational modifications of histones in human sperm

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We examined the levels and distribution of post-translationally modified histones and protamines in human sperm. Using western blot immunoassay, immunofluorescence, mass spectrometry (MS) and FLIM-FRET approaches, we analysed the status of histone modifications and the protamine P2. Among individual samples, we observed variability in the levels of H3K9me1, H3K9me2, H3K27me3, H3K36me3, and H3K79me1, but the level of acetylated (ac) histones H4 was relatively stable in the sperm head fractions, as demonstrated by western blot analysis. Sperm heads with lower levels of P2 exhibited lower levels of H3K9ac, H3K9me1, H3K27me3, H3K36me3, and H3K79me1. A very strong correlation was observed between the levels of P2 and H3K9me2. FLIM-FRET analysis additionally revealed that acetylated histones H4 are not only parts of sperm chromatin but also appear in a non-integrated form. Intriguingly, H4ac and H3K27me3 were detected in sperm tail fractions via western blot analysis. An appearance of specific histone H3 and H4 acetylation and H3 methylation in sperm tail fractions was also confirmed by both LC-MS/MS and MALDI-TOF MS analysis. Taken together, these data indicate that particular post-translational modifications of histones are uniquely distributed in human sperm, and this distribution varies among individuals and among the sperm of a single individual.

Reference

J. Krejčí, et al., Post-Translational Modifications of Histones in Human Sperm. J Cell Biochem. 2015 Mar 23. doi: 10.1002/jcb.25170.

48 3D Electron and Confocal Microscopy Reveals New Structure of Odontoblasts during Development

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Odontoblasts are cells which form dentin, one of three hard matrixes of teeth. These highly specialized cells possess long processes leading through dentin layer and remaining in of dentinal tubules for lifetime. Odontoblastdentin complex has very characteristic features and detailed knowledge of its structure is crucial for understanding its functional and developmental consequences.

In our study we combined different 3D imaging techniques including Focused Ion Beam Scanning Electron Microscopy (FIBSEM) and confocal imaging of genetically traced non-decalcified adult mice incisors to elucidate a detailed structure of odontoblasts during maturation. Our results show distinct morphological features of odontoblasts during different stages of development and explain mechanisms of formation of the main odontoblast process and terminal branching near Dentin-Enamel Junction (DEJ). We also demonstrate numerous, previously unanticipated, contacts between odontoblasts and processes of dental pulp cells. On the basis of our data we suggest a new model of odontoblast-dentin complex development and structure.

49 Comparison of the effect of different molecular weight hyaluronan from two different sources on mouse macrophages activation

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Glycosaminoglycan hyaluronan (HA) is an abundant component of the extracellular matrix. Current studies suggest that the direct effects of HA on cells are dependent on its molecular weight (MW), as low MW HA exhibits immunostimulatory activity compared to high MW HA. However, significant variations are reported among various authors. These differences in observed HA activity could also be connected with the employed HA preparation, particularly with its purity and origin. Thus, here, we compared the activation of mouse immune cells by HA preparation from rooster comb (100 kDa, 500 kDa, 997 kDa) and Streptococcus equi biotechnological origin (71 kDa, 500 kDa, 1000 kDa) of precisely defined MW. Interestingly, in contrast to established dogma, only high and middle size MW HA preparations of both microbial and animal origin induced TNF-α release from mouse macrophage cell line RAW 264.7 and bone marrow-derived macrophages (BMDM). This effect was generally more significant for HA of animal origin. Similar trends were observed employing isolated mouse splenocytes and mouse whole blood. All tested HA preparations also induced ROS production by isolated splenocytes; however, this effect was independent of the MW and origin of HA. All HA preparations failed to induce expression of cyclooxygenase-2 and inducible NO synthase and production of NO in mouse macrophages cell lines RAW 264.7 and BMDM and to induce ROS production in mouse whole blood. Data show significant differences among the effects of HA of animal and microbial origin on immune cells in vitro together, with variable HA effects on various well established parameters of immune cell functions.

50 Blood Coagulation and Platelet Adhesion on Polyaniline Films

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Polyaniline (PANI) belongs to the polymers with unique properties, mainly conductivity, with broad application potential in biomedicine [1]. As a hemocompatibility is one of the major biocompatibility parameters, in this study the PANI was modified with a biological active acid influencing hemostasis, namely poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPSA) [2]. The changes in blood coagulation and platelet adhesion induced by the different PANI surfaces were investigated. The anticoagulation activity was determined via monitoring the following coagulation parameters: thrombin clotting time (TCT); activated partial thromboplastin time (aPTT); and prothrombin time (PT). The interaction with coagulation factors X, V and II was also studied.

The PANI-emeraldine has no influence on coagulation either platelet adhesion. However, modification with PAMPSA has influenced it. When the PANI surface was reprotonated with PAMPSA solution coagulation was still under physiological conditions, while the platelet adhesion has notably decreased. Nevertheless, by adding PAMPSA to the PANI reaction mixture, the platelet adhesion significantly decreased and in addition the coagulation has been completely inhibited.

Whereas that pristine PANI films loses their conductivity at pH 4.5, also the UV-Vis spectra of the PANI-PAMPSA was measured to evaluate the pH of the transition between conducting PANI–PAMSA salt and its non-conducting, deprotonated base. It was demonstrated that PANI-PAMPSA showed improved pH stability with the transition at pH of 6 what opens a new possibility for PANI applications in biomedical field.

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References
[1] S. Nambiar, J.T.W. Yeow. Biosensors and Bioelectronics. 26, 1825–1832 (2011)
[2] R.M. Moura, A.A. Alencar de Queiroz. Artificial Organs, 35, 471-477 (2011).

51 The role of HIF-1alpha in the regulation of cardiomyogenesis in vitro

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Cardiomyogenesis is controlled by interaction between signaling pathways, transcription factors and cell microenvironment. It is known that hypoxia modulates in vitro differentiation of pluripotent cells into the cardiomyocytes through HIF-1alpha dependent pathways. However, the role of HIF-1alpha stabilization in the regulation of murine embryonic stem cells (mESC) differentiation is not precisely understood. Thus, with the aim to clarify the role of HIF-1alpha in cardiac progenitor cell programming and cardiomyocyte formation and maturation, cardiomyogenesis in wild type and HIF-1alpha deficient mESC was analyzed in vitro.

Interestingly, natural stabilization of HIF-1alpha in wild type cardiomyocytes was observed. The expression of cardiac markers was similar comparing wild type and HIF-1alpha deficient cells during the early phase of differentiation up to day 5. In contrast, in the late phase (days 10 and 15), cardiac markers were significantly lower in HIF-1alpha deficient cells. Moreover, HIF-1alpha deficiency was associated with significantly higher expression of neuronal markers and lower number of beating foci in HIF-1alpha deficient differentiated cells. However, at the end of differentiation process, both wild type and HIF1alpha deficient cardiomyocytes revealed similar intracellular localization of the contractile proteins.
Our results suggest that HIF-1alpha deficiency negatively regulates spontaneous cardiomyogenesis while promotes neurogenesis in differentiating mESC in vitro.

52 Plasma synthesized iron oxide nanoparticles with outlook to potential application in MRI

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The maghemite (g-Fe_2O_3) nanoparticles are well established magnetic resonance imaging (MRI) contrast-enhancing agent and some commercial products based on them are approved for a clinical use in humans. However, current preparation methods are mostly based on error-prone and time-consuming series of wet-chemical steps.

We report on iron oxide based nanoparticles synthesized in single step process by plasma enhanced chemical vapor deposition (PECVD) using low-pressure microwave discharge from iron pentacarbonyl $Fe(CO)_5$ precursor. Depending on operating conditions (excitation power, precursor flow, buffer gas flow) it can produce [1] nanoparticles with different dimensions and chemical composition. Their physical properties were analyzed using XRD, SEM, AFM and Raman spectroscopy. Moreover, the Atomic Force Microscope (AFM) Bruker Dimension Icon was used for 3D characterization of iron oxide particles with sub-nanometer resolution. The data were analyzed using Gwyddion [2] software. The AFM measurements show that size of the particles varies from nano- up to micro- scale. Size distributions of the particles can be directly calculated from AFM images. Topographic height profiles of nanoparticles and shape characterization bring deeper understanding of particles morphology.

The pilot test of MRI suitability of the plasma prepared nanoparticles was carried out in agar gel at 4.7 T with a spin echo sequence. The effect of maghemite nanoparticles was clearly exhibited. However, the surface and colloid properties of the single step produced nanoparticles were suboptimal.

References
[1] O. Jašek et al., Proceedings of Nanocon (2010) 487
[2] D. Nečas, P. Klapetek Cent. Eur. J. Phys. 10 (1) (2012) 181

53 High resolution micro-CT of low attenuating material utilizing large area photon counting detector

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Utilizing of pixelated detectors in radiography and micro-CT measurements provides adequate solution of problems with low attenuating material imaging. One of such material is porous Gellan Gum reinforced with bioactive glass. This synthetic biomaterial is used for tissue engineering (scaffolding), highly optimized for its biocompatibility, bioresorbability, porosity and mechanical properties. Tomographic control of the material behavior under the mechanical stress is valuable method for the study of these properties. Inspected sample with porosity almost 90% has extremely low X-ray transmission attenuation.

Used world largest photon counting X-ray imaging detector WidePIX is composed of a matrix of 10x10 tiles of silicon pixel detectors Timepix (each of 256 x 256 pixels with pitch of 55 microns) having fully sensitive area of 143 x 143 mm without gaps between tiles. Comparison of micro-CT reconstructions of bone scaffolds and other biological material achieved with photon counting detector and conventional flat panel will be presented.

References

[1] Jakubek, J., Jakubek, M., Platkevic, M., Soukup, P., Turecek, D., Sykora, V., Vavrik, D. Large area pixel detector WIDEPIX with full area sensitivity composed of 100 Timepix assemblies with edgeless sensors. (2014) *Journal of Instrumentation*, 9(04), pp. C04018-C04018. DOI: 10.1088/1748- 0221/9/04/c04018.
[2] Gantar, A., Da Silva, L. P., Oliveira, J. M., Marques, A. P., Correlo, V. M., Novak, S., Reis, R. L. Nanoparticulate bioactive-glass-reinforced gellan-gum hydrogels for bone-tissue engineering. (2014) *Materials Science and Engineering*: C, 43, pp. 27-36. DOI: 10.1016/j. msec.2014.06.045.

54 Biodegradable Elastic Polyurethane Films Applicable as Vascular Grafts

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Biodegradable polyurethane (bio-PU) films for medical applications were synthesized newly by "green" polymerization method without the use of potentially toxic organic solvent. Bio-PU samples were prepared from both hydrophobic polycaprolactone (PCL) and hydrophilic polyethylene glycol (PEG) with different PCL/PEG molar ratios to evaluate their chemical, physical and biological properties. Abrupt enhancement of mechanical properties reaching strain at break of 900 % was observed at samples when PCL/PEG ratio was higher than 3. As presupposed, swelling and hydrolytical degradation increased with the amount of hydrophilic PEG. Bio-PU sample made only of PEG polyol exhibited ≈ 78 % of water content within 1 one day of swelling and degraded completely within 5 months in contrary to bio-PU made only of PCL polyol, which absorbed only 2 % of water within one day and has shown good stability after a period of one year. Therefore, different physical properties meeting certain application could be adjusted by the change in PCL/PEG ratio. Three representatives from prepared bio-PU samples were chosen for preliminary tests of human mesenchymal stem cells (MSCs) response to the material. As a result, MSCs have not adhered on bio-PU samples with high PEG content. However, samples with high abundance of PCL allowed good cell adhesion on the surface, but promoted detachment of the MSCs from the surface after one week. Detected low adherence of cells to the bio-PU surface predestines the material for the use as an artificial vein or vascular grafts.

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55 Perturbation of 3R/4R Tau Ratio Impairs APP Axonal Transport in Neurons Derived from Human Embryonic Stem Cells (hESC)

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by senile plaques and neurofibrillary tangles. Human Embryonic Stem Cells (hESC) research may provide an opportunity to investigate novel approaches to the understanding of the pathophysiology of Alzheimer's disease (AD). Here we use hESC to investigate tau protein, whose imbalance has been associated with (AD). We hypothesize that the perturbation in the physiological ratio of 3R/4R tau could trigger impaired axonal transport thereby contributing to the pathophysiology of AD.

We have derived human neurons from hESCs and induced a 3R/4R tau imbalance by using a spliceosome-mediated RNA trans-splicing system (SMaRT). Cell cultures with predominant 3R or 4R tau expression exhibited impaired axonal transport compared with control. More specifically, there was a significant reduction of average velocity and segmental velocity distribution. Intriguingly analyses of all parameters of axonal transport indicated consistently significant impairments in anterograde axonal transport with milder effect on retrograde axonal transport.

Overexpression of tau does not represent the optimal, physiological setting to test axonal transport related biological questions, since it does not allow to discriminate whether axonal changes are the result of overexpression or changes in tau. Furthermore, and unlike APP, tau is not overexpressed in tau related disorders. Perturbing 3R/4R tau ratio represents a novel, more physiological approach to testing the biology of tau isoforms. Here we show that subtle changes in the tau ratio cause significant impairments in axonal transport of APP.

References

[25] Zhang XQ, Zhang SC., Methods Mol Biol. 2010; 584:355-66.
[26] Avale ME et al. Human Molecular Genetics, 2013; Vol. 22, No. 13 2603–2611
[27] Falzone TL et al. The Journal of Neuroscience, 2009; 29 (18):5758-5767
[28] Stokin GB et al. Science, 2005; 307, 1282-1288

56 Accuracy of Digital Impressions and Fitness of Single Crowns Based on Digital Impressions

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In this study, the accuracy (precision and trueness) of digital impressions and the fitness of single crowns manufactured based on digital impressions were evaluated. #14-17 epoxy resin dentitions were made, while full-crown preparations of extracted natural teeth were embedded at #16. (1) To assess precision, deviations among repeated scan models made by intraoral scanner TRIOS and MHT and model scanner D700 and inEos were calculated through best-fit algorithm and three-dimensional (3D) comparison. Root mean square (RMS) and color-coded difference images were offered. (2) To assess trueness, micro computed tomography (micro-CT) was used to get the reference model (REF). Deviations between REF and repeated scan models (from (1)) were calculated. (3) To assess fitness, single crowns were manufactured based on TRIOS, MHT, D700 and inEos scan models. The adhesive gaps were evaluated under stereomicroscope after cross-sectioned. Digital impressions showed lower precision and better trueness. Except for MHT, the means of RMS for precision were lower than 10 μm. Digital impressions showed better internal fitness. Fitness of single crowns based on digital impressions was up to clinical standard. Digital impressions could be an alternative method for single crowns manufacturing.

57 "FILTERING" INTERMOLECULAR NOES IN A 50 KDA RNA-PROTEIN COMPLEX BY COMBINING PROTEIN PERDEUTERATION WITH SELECTIVE, AMINO ACID-TYPE AND RNA LABELING

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Protein interactions with mRNA regulate gene expression post-transcriptionally from mRNA splicing to mRNA transport and translation. TDP-43 (TAR DNA binding protein - 43 kDa) binds in two copies to a UG-repeat sequence upstream of exon 9 of CFTR (cystic fibrosis transmembrane conductance regulator) and this leads to skipping of the exon associated with severe forms of cystic fibrosis. In principle, NMR spectroscopy is a powerful tool to determine the structure of such regulatory RNA-protein interactions, but structure determination of larger RNA-protein assemblies still remains challenging. Especially the detection and assignment of intermolecular contacts is difficult in larger RNA-protein complexes (50kDa) since 2D and 3D filtered/edited NOESY experiment suffer from short transverse relaxation times.

We therefore eliminate the need for lengthy filtering and editing elements by using selective amino acid-type labeling (both ^{13}C,^{15}N-labeled and unlabeled) in a fully deuterated protein background. Simple 2D NOESY spectra recorded on fully deuterated TDP-43 with selected protonated amino acids in complex with unlabeled RNA yield intermolecular NOEs between amino acid sidechains and RNA ribose and base protons with high sensitivity. Similarly, 3D 13C-edited HMQC NOESY spectra acquired on fully deuterated TDP-43 with selected ^{13}C,^{15}N-labeled amino acids or 13C ILV-labeling in complex with unlabeled RNA allows unambiguous NOE assignment of amino acid sidechains to RNA protons. To confirm the assignments of RNA ribose and base proton NOEs to the protein sidechains, we record 3D ^{13}C-edited NOESY HSQCs using fully or segmentally ^{13}C-labeled RNA in complex with fully deuterated TDP-43 with selected, protonated amino acids. Using this array of labeling schemes, we could recover over 600 intermolecular NOEs between the two TDP-43 copies and the UG-rich RNA. Our approach should also enable structure determination of other large RNA-protein complexes. The preliminary structure will be presented and the implications for splicing regulation will be discussed.

58 Numerical simulation of liver perfusion

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We use a collection of Python programs for numerical simulation of liver perfusion [1, 2]. We developed an application [3] for semi-automatic generation of a finite element mesh of the human liver from computed tomography scans and for reconstruction of the liver vascular structures. When the real vascular trees can not be obtained from the CT data we generate artificial trees using a constrained constructive optimization method. The generated FE mesh and vascular trees are imported into SfePy (Simple Finite Elements in Python) [4] and numerical simulations are performed in order to get the pressure distribution and perfusion flow in the liver tissue. The model of contrast fluid propagation provides time-dependent concentration of the tracer that can be compared with the standard medical measurements. It will allow us to solve the inverse problem in order to identify some of the perfusion parameters of our models.

References

[29] E. Rohan, V. Lukeš, A. Jonášová, O. Bublík. Towards microstructure based tissue perfusion reconstruction from CT using multiscale modeling. In Proc. of the 10th World Congress on Computational Mechanics, Sao Paulo, Brasil, 2012.
[30] V. Lukeš, M. Jiřík, A. Jonášová, E. Rohan, O. Bublík, R. Cimrman. Numerical simulation of liver perfusion: from CT scans to FE model. In Proc. of the 7th European Conference on Python in Science (EuroSciPy 2014), pages 79-84, 2014. http://arxiv.org/pdf/1412.6412
[31] V. Lukeš. DICOM2FEM – application for semi-automatic generation of finite element meshes. Home page: http://sfepy.org/dicom2fem
[32] R. Cimrman. SfePy - Write Your Own FE Application. In Proceedings of the 6th European Conference on Python in Science (EuroSciPy 2013), pages 65-70, 2014. http://arxiv.org/abs/1404.6391

59 Biomaterial for optical sensing of glucose based on organic-inorganic polymer

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Organic-inorganic polymer Ormocer® is a hybrid polymer material synthesized by the sol-gel process, which is cured by UV radiation. In addition to curing at low temperatures chemical and thermal stability, final properties of Ormocer® as toughness and gas permeability might by modified in broad ranges. Currently Ormocer®´s are used in dental medicine and ophthalmology. We tested this polymer as an encapsulation carrier of biorecognition layer of optical biosensor of glucose.

The biorecognition layer, the biomaterial, contained enzyme glucose oxidase prepolymerized on Sepabeads® and ruthenium complex, which were both incorporated in Ormocer®. We studied the influence an amount of Ormocer® KSK 1238 on analytical features of the biosensor as sensitivity (SE), limit of detection (LD), limit of quantification (LQ), linear dynamic range (LDR) and long-term stability.

We found that biorecognition layers containing less than 50 % of Ormocer® were mechanically unstable. The layers that included 65 % of Ormocer® performed higher sensitivity (10 %) and lower LD, LQ, and LDR (15%) in comparison with these comprising 80% of Ormocer®.

The analytical features of the biosensors were stable with the relative standard deviation 10 %, during one year.

Chemical inertness and ruggedness of the biomaterial of the biorecognition layer make possible to use this sensor in industrial bioreactors as well as in human medicine.

60 Detection of mercury contamination in soil by bioluminescent bioreporter

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Escherichia coli ARL1 is genetically modified microorganism, which produces bioluminescence in presence of mercury ions. The aim of this study was to find out convenient conditions of bioluminescence induction for detection of mercury in soil.

We monitored bioluminescence *of E. coli* ARL1 induced by soils and soils extracts; both acid and alkaline extracts. The soil, from a locality in the Czech Republic, was contaminated with 382 ng g^{-1} of mercury and Certified Reference Materials contained 78.5±10.3 resp. 171±16 ng g^{-1} of mercury. Intensities of bioluminescence were measured in 12 and 96 well plates, using multi-well reader Omega.

Soils in phosphate buffer induced *E.coli* ARL1 bioluminescence comparable with the background, due to losses of emitted light. These were results of light dispersion and absorption in soils suspension. Higher intensities of bioluminescence were achieved by acceleration of mass transfer by shaking of plates during the bioluminescence measurement.

The soils were extracted with 0.2M HCl and 0.2M NaOH for 12 hours. pH of each extract was adjusted to pH 7 before addition of cell suspension. Further increase of bioluminescent intensity was achieved by pH optimization.
The developed bioanalytical procedure is proposed for semiquantitative detections of mercury in soils in concentration order ng g^{-1}.

References
[1] Dahl, AL., Sanseverino J. & Gaillard J., (2011) Bacterial bioreporter detects mercury in the presence of excess EDTA. Environmental Chemistry, 8(6), 552-560. http://dx.doi.org/10.1071/EN11043

61 Plasma polymer coatings for the development of effective QCM and SPR immunosensors

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Immunosensors with highly sensitive and rapid detection capabilities of various biomolecules are of a great demand in the field of biomedicine and environmental control. The two mostly employed transducer principles are optical surface plasmon resonance (SPR) and mass sensitive quartz crystal microbalance (QCM). The first task encountered in the immunosensor development is the preparation of a coating matrix suitable for the immobilization of antibody on the golden layer. The most popular approaches are based on wet chemical treatments, the formation of self-assembled monolayers (SAMs) of alkanethiols and disulfides, or polyethylenimine (PEI). However, these approaches suffer from several drawbacks such as a poor stability, long time preparation, unstable baseline or a high level of noise.

As an alternative to SAMs, the deposition of thin functional coatings by plasma polymerization can be employed. The plasma polymerization has already been successfully applied to the deposition of thin films containing carboxyl, amine, anhydride groups. It is known that the essential chemical and thickness stability of the plasma layers can be achieved by tuning the plasma parameters.

In this work the Plasma Enhanced Chemical Vapor Deposition (PECVD) method, which is energy efficient and environmentally friendly dry process, was employed for the deposition of thin stable coatings onto the surfaces of QCM and SPR chips.
In case of QCMs the pulsed plasma polymerization of cyclopropylamine (CPA) was used to obtain the stable amine-rich polymer using capacitively coupled radio frequency discharge. Then three different antibody immobilization approaches were studied and compared.

In case of SPR sensors the pulsed plasma co-polymerization of acetylene and maleic anhydride (MA) was employed for the synthesis of stable carboxyl-rich thin films (dielectric barrier discharge) and various antibody immobilization approaches were also tested.
For the developed immunosensors the stable baseline before and after the interactions with the antigen was recorded. Selective and high response was achieved during the reaction with the solution of antigen. The results confirmed that the presented methodologies for the grafting of biomolecules on the gold surfaces have great potential for biosensing applications.

62 Critical role of nitro-oleic acid in polarization of different macrophage subpopulations and fibrosis development

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Nitro-fatty acids are currently suggested as highly promising endogenous compounds for treatment of diseases associated with deregulated immune homeostasis. These pathological processes are, at least partially, caused by changes in macrophage polarization into specific pro-inflammatory or regulatory subsets. The main goal of our experiments was to characterize the role of nitro-oleic (OA-NO_2) acid in LPS- and IL-4- activated macrophages to reveal the specific signalling mechanisms, which are potentially responsible for OA-NO_2modulation of inflammation and fibroblast/myofibroblast transdifferentiation.

We show that OA-NO_2 inhibits endotoxin-stimulated production of both pro-inflammatory and immuno-regulatory cytokines, NO and superoxide production. Moreover, OA-NO_2 acid is able to effectively down regulate the IL-4-mediated immune response of macrophages by inhibition of Arg-I expression and TGF-β production. These effects are mediated via down regulation of STATs and MAPKs activation. Importantly, OA-NO_2 is capable to effectively diminish the fibrotic process, what was verified *in vivo* using cardiac fibrosis model.

Our study provides the unique results showing the protective effects ofOA-NO_2 in pro-inflammatory and regulatory macrophages that play an important role in regulation of chronic inflammation and fibrosis.

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63 Investigating protein conformational dynamics using computational modeling and analysis of protein structures

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Protein structure/function relationship is the complex one. Ideal solution leading to understanding of the whole interplay between atoms involved in protein structure is still behind the horizon of today science. Fortunately growing body of information of protein structures and their behavior shed the light on the problem and enables us to split the issue to smaller parts more accessible to our study and understanding. In our case the simplification used in the study is focused on finding parts of protein with higher flexibility on the background of more rigid structures. The effort is made to associate these flexible structures to protein functions mainly in gating properties into protein interior. Recent studies show high importance of protein interior structures like tunnels and channels for the functions of e. g. enzyme active sites or receptor ligand binding sites. The question of finding gating residues and structure elements remain to large extent unresolved mainly due to prevalence of static protein structures in protein databases.

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64 Preparation of hydroxyapatite/silk fibroin/collagen composites

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Biomaterials used for bone regeneration to mimic natural bone structure should contain organic and inorganic parts. Natural bone is composed of type I collagen and nanohydroxyapatite crystals [HA, $Ca_{10}(PO4)_6(OH)_2$] [1]. The aim of this work was to prepare silk fibroin/collagen 3D composites. These two proteins were mixed with different weight ratios. Scaffolds were obtained using lyophilisation process. Then all samples were modified with EDC/NHS. Hydroxyapatite was precipitated by immersion of scaffolds in 200 ml of 200 mM $CaCl_2$/Tris–HCl solution (pH 7.5). After 1 min scaffolds were rinsed with deionized water for 15 s and places in to 200 ml of 120 mM Na_2HPO_4/Tris-HCl solution (pH 7.5) for 1 minand again washed with deionized water. Scaffolds were dried in a desiccator containing calcium chloride [2]. Microstructure of composites was tested by using scanning electron microscope (SEM) and the element content in samples was checked by energy dispersive X-ray spectrometer (EDX). In lyophilisation process 3D porous material based on the blends of silk fibroin and collagen can be obtained. It was possible to precipitate hydroxyapatite in the polymer matrix and 3D composites were obtained.

References

[33] Y. Li, Y. Cai, X. Kong, J. Yao, Appl. Surf. Sci., 255, (2008) p. 1681.
[34] D.G.T. Strange, M.L. Oyen, Acta Biomaterialica, 7, (2011) p. 3586.

65 Functional Biodegradable Hydrogels End-Linked by Blue Light

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Temperature-sensitive biodegradable PLGA–PEG–PLGA hydrogels functionalized by itaconic anhydride (ITA) with reactive double bonds and carboxylic acid groups were synthesized by ring opening polymerization. Modification by ITA extends possibility of hydrogels application. Due to the double bonds, hydrogels can be chemically crosslinked by commercially available blue light used in dental medicine without further crosslinker in order to obtain end-linked hydrogels. In addition to that, –COOH groups can be used for coupling with bioactive materials (drugs or proteins) The resulting hydrogels with controlled lifetime and drug release are more stable, and therefore degrade more slowly. These properties are particularly preferred for temporary orthopedic implants, which gradually degrade during the healing and growth of human bones. The healing process can be also accelerated by release of drugs. Crosslinking efficiency by blue light and hydrolytical stability in ultrapure water increased with increasing amount of bonded ITA (functional groups) and with crosslinking time. End-linked hydrogels are able absorb large amount of water. Influence of amount of bonded ITA on swelling behavior of crosslinked samples is shown at figure 1.

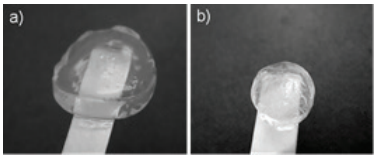

Figure 1: End-linked ITA/PLGA-PEG-PLGA/ITA hydrogels with 37 % ITA (a) and 63 % ITA (b) in swelled state at 11th day of swelling.

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66 Mechanisms of human embryonic stem cells response to alterations of endoplasmic reticulum homeostasis

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Endoplasmic reticulum (ER) represents, in addition to the membrane biosynthesis, storage of calcium ions and number of metabolic processes, a default organelle for synthesis and posttranslational modification of proteins. ER also represents a significant role in the homeostasis of a cell. The cell can react to failure of homeostasis of ER by several mechanisms. The most significant response to ER stress is UPR (unfolded protein response), which is associated with the change of expression of molecular chaperones, especially CHOP and BiP. Depending to the ER stress inductor, UPR pathway may provide cell adaptation, or it may provide transition of the cell to apoptosis. The mechanism of ER stress was described in detail in various somatic and cancer cell lines, however, in human pluripotent stem cells the information is still incomplete.

In this work, we have developed a model for ER stress study in hESCs by inhibiting N-glycosylation allowing controlled activation of proapoptotic and adaptation UPR branches. On the basis of individual experiments, the general mechanism as well as differences among individual hES cell lines of the same type were identified. The differences between the cells cultured in a medium without tunicamycin and cells induced by tunicamycin were found on both the levels of gene and protein expression. Also significant differences in morphology were identified. Knowledge of mechanisms by which hESCs respond on suboptimal conditions, contributes to the development of safer culture protocols of manipulation with pluripotent cells in vitro as well as to further development of knowledge of early embryogenesis. Intermediate knowledge may also be useful in the future in the field of regenerative medicine.

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67 Investigation of plasma treated alumina powders by chemiluminescence

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Sintering of plasma treated alumina powder was found to improve the microstructure of final ceramic body [1]. So far the mechanism responsible for this improvement is not fully understood, therefore further investigation of the plasma induced changes is needed. Recently, we have observed that plasma treatment of the alumina powder results a significant increase of thermally stimulated luminescence signal. The aim of this work was to identify the particular process responsible for the produced luminescence. For this purpose we did a series of experiments, in which the samples were heated at the constant heating rate and the emitted luminescence was detected by chemiluminescence detector. Our results indicate that the luminescence arises from thermoluminescence - thermal detrapping of electrons excited to trapping states by plasma action. It was also found that the preceding thermal annealing can greatly influence the resulting thermoluminescence curves; and that the UV radiation does not play a crucial part in the excitation of the electrons to the trapping states.

References

[35] Z. Szalay et al., Ceramics International, 40, 8, 12737-12743, 2014.

68 The influence of shape and size of HA nanoparticles on robocast 45S5 scaffolds coated with HA-PCL composites

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The 45S5 bioglass is highly bioactive and there are many reports on its application in bone tissue engineering. Hiring 45S5 in robocasting has been done recently, providing a means to improve the mechanical performance of scaffolds by a much greater level of control over pore architecture and more regular strut morphologies. In spite of that, the main weak point of 45S5 scaffolds still lies in their intrinsic brittleness and the poor mechanical resistance associated to their porosity. One applicable solution for overcoming brittleness of 3D scaffolds is the addition of a polymer phase, which would fill micropores and cracks on the strut surfaces. Such a bioactive glass-polymer composite structure would combine the advantages of a polymer with those of an inorganic bioactive phase, potentially leading to a structure exhibiting higher stiffness, fracture strength and toughness [1].

In this work nanocomposite coatings consisting of PCL as a polymer matrix and nano-HA as ceramic filler were achieved by dip-coating of 45S5 bioglass robocast scaffolds. The effect of the shape and size of nano-HA particles on the mechanical properties of composite scaffolds were evaluated [2].

References
[36] S. Eqtesadi, A. Motealleh, A. Pajares, F. Guiberteau, P. Miranda, Journal of the European Ceramic Society, 2015, In Press.
[37] S.I. Roohani-Esfahani, S. Nouri-Khorasani, Z. Lu, R. Appleyard, H. Zreiqat, Biomaterials,31, 2010, 5498-5509.

69 The Hippo Signaling Pathway in Adipose Tissue-Derived Mesenchymal Stem Cell Maintenance and Differentiation

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Chemical and mechanical stimuli provided from the extracellular milieu are known to regulate cell behavior and stem cell fate decisions and therefore contribute to organ development, tissue remodeling, or degenerative disease progression. YAP and TAZ, the key effector proteins of the Hippo Pathway, act as cellular mechanotransducers, integrating the mechanical cues from the extracellular matrix (ECM) and the surrounding cells into biochemical intracellular signals in order to control cell migration, proliferation and differentiation. Their activity as transcriptional co-activators has been associated to their ability to interact with cell- specific transcription factors in the nucleus. Here, we take advantage of micropatterned surfaces and loss-of-function techniques to study the effects of YAP/TAZ activity in human mesenchymal stem cells derived from adipose tissue (AD-MSCs) at a single cell level. Our results show that YAP/TAZ nuclear localization is controlled by the availability of cell-ECM or cell-cell binding sites. In particular, we show that cadherins act as upstream regulators of the Hippo pathway in AD-MSCs by controlling YAP/TAZ relocation from the nucleus to the cytoplasmic compartment, where the proteins are inactivated and destroyed. YAP and TAZ appear to interact with focal adhesions, cytoskeletal assembly, and tension distribution in AD-MSCs. Importantly, although YAP and TAZ have been considered proteins with homologous functions, their knockdown differently affects cell size and shape, while revealing distinct clusters of target genes for the two paralog proteins. Together with the evidence that YAP and TAZ could exert differential roles during AD-MSC differentiation towards the mesodermal lineage, these results suggest novel activities for these two mechanosensors in mesenchymal stem cells.

70 Determination of binding constant by capillary electrophoresis - frontal analysis and isothermal titration calorimetry

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Strength of the binding between ligands (drugs) and proteins is commonly described by binding constant. These interactions have a significant effect on the biological activity, pharmacodynamics and pharmacokinetics properties of drugs. Therefore these processes are studied and tested in the development of new drugs [1].

In this study, the binding constant was determined by two methods: capillary electrophoresis – frontal analysis (CE FA) and isothermal titration calorimetry (ITC). Both CE FA and ITC measure the affinity of binding partners in their native states without immobilization or labelling of the interacting partners. What is more we can simulated physiological conditions because the investigated interactions takes place in a solution. Disadvantages of ITC are a large sample consumption and a problem with estimation of week affinity interactions [2], the advantage of CE-FA are a very low sample consumption and a high resolution and also it does not require the highly purified samples.

The main objective of this study was to compare both these methodological approaches for the study of drug-protein interaction. The couple of diclofenac and HSA was chosen as a model system.

References
[38] Sharma, R., Choudhary, S., Kishore, N., European Journal of Pharmaceutical Sciences 46 (2012) 435.
[39] Vuignier, K., Schappler, J., Veuthey, J. L., Carrupt, P. A., Martel, S., Analytical and Bioanalytical Chemistry 398 (2010) 53.

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71 Function of cyclin-dependent kinase 13 during mouse development

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Gene expression is a tightly controlled process involved in a broad spectrum of biological processes, ultimately giving cells the ability to take control of their growth, apoptosis, differentiation and developmental potential. Regulation of gene expression is orchestrated primarily at the level of transcription of specific mRNAs by RNA polymerase II (RNAPII) and its activity is primarily regulated at the level of posttranslational modifications; among them, phosphorylation is the most prevalent. We have recently unveiled that the cyclin-dependent kinase 13 (CDK13), similar to CDK9 and CDK12, phosphorylates RNAPII and thereby influence transcription of various sets of genes involved in variety of physiological processes. Importantly, the function of CDK13 during mouse development has not been demonstrated as of today, therefore we decided to dissect functional role of CDK13 during early mouse development. Initially, we employed embryonic stem cells with potential to generate complete and conditional knock-out CDK13 mice. Heterozygous CDK13+/- mice were bred and their offsprings with wild type allele were born at regular rates and appear normal; however, CDK13 complete knock-out mice were not born at all. It shows that CDK13 plays the key function during early mouse development. At the moment, we carry out detail analyses to reveal at what embryonic stage the lethal phenotype appears.

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72 Macroporous calcium phosphate scaffolds with enhanced mechanical properties

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Developing of ceramic artificial bone grafts is becoming a promising approach for the repair of large bone defects. The ideal scaffold should be biocompatible, mechanically stable and highly porous. Ceramics based on calcium phosphates (Ca–P) exhibit excellent bioactivity and bone bonding ability but their application in real systems is limited due to low fracture toughness and strength. Hence, the aim of this work was to prepare mechanically strong scaffolds with maintaining the bioactivity of calcium phosphate ceramics.

Silica reinforced calcium phosphate porous scaffolds were prepared via polymer replica technique, the technique that best mimics the macrostructure of trabecular bone. Scaffolds with different amount of silica (5-20 wt%) exhibited well-interconnected open pore structure with pore size ranging from 150 to 600 μm. The apparent porosity after sintering, mainly affected by the viscosity of the coating suspensions, reached 70-95%. The silica reinforced Ca-P scaffolds exhibited higher strength than the Ca-P scaffolds of the same porosity.

The biochemical evaluations of scaffolds were conducting by in vitro testing methods. The apatite formation appeared on the surface of coated scaffolds after immersion in simulated body fluid (SBF) indicated a sufficient bioactivity of prepared samples. According to ISO 10993/5 neither of tested ceramics induced any adverse effect on MG63 cells. Tested materials were cytocompatible and could be suitable candidates for clinical applications.

73 Modelling the chemistry in a helium/air plasma jet for bacteria inactivation

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Atmospheric-pressure plasma jets (APPJs) ignited in a noble gas and operating in ambient atmosphere have been examined in the past years in relation to a number of promising biomedical applications including wound healing, surface sterilization or cancer treatment. The low-temperature plasma affects living tissue through a synergy of several phenomena, most importantly the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the presence of transient electric fields. Despite numerous experimental studies, the mechanism of interaction of APPJs with biological systems still remains poorly understood. To provide deeper insight into this mechanism, we devised a numerical model describing the production, transport and surface loss of various reactive species (e.g. O, OH, NO_x) in the afterglow of an APPJ. The results of this model are correlated with investigations of *E.coli* inactivation by this plasma jet, the efficacy of which depends on the operating parameters of the plasma jet. By doing so, we aim to identify which reactive species play an important role in this application. It is shown that the gas dynamics in the plasma jet has a dramatic influence on the reactive species transport in the plasma afterglow and does, therefore, influence the *E.Coli* inactivation patterns.

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74 Cell mechanosensors as regulators of cardiac pathologies

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Cell mechanosensors have been recently described as key determinants in cell fate and function. In fact, they can be stimulated by the mechanical forces arising from dynamical modification of the extracellular matrix (ECM) composition and lead to the activation of multiple classic signaling pathways.

Their activity in regulating cardiac contractile function has been recently highlighted. Following changes in ECM components and subsequent modification in intracellular signaling, mechanosensors such as those controlled by the Hippo pathway or those triggered upon Integrin's activation have been found altered and/or impaired in a number of pathologies such as cardiac hypertrophy or infarction.

For instance, YAP/TAZ expression is triggered in cardiomyocytes at the infarction border zone and *vinculin* mutations are identified in patients with *dilated cardiomyopathy*.
However, the functional relevance of the mechanosensing alterations in the onset and development of these diseases is still poorly understood. With the aim to highlight the contribution of tissue-specific mechanosensors to cardiac pathologies, our group has started an integrative study to correlate ECM topography and 3D nanostructure with the differential distribution of mechanosensors.

As a first approach, the pattern of expression of mechanosensors in cardiac biopsies from healthy donors has been evaluated. Decellularized matrices from healthy and infarcted mice hearts have been used in parallel, as a tool to assess ECM remodeling. Preliminary analysis of protein rearrangement in decellularized matrices revealed that drastic structural changes occur as a consequence of myocardial infarction.
Here we explore heart ECM remodeling and differential distribution of mechanosensors as a starting point to establish a functional network towards a better understanding of cardiac pathologies.

75 Expression of fibroblast surface markers in permanent epithelial cell lines undergoing EMT

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When working with human tissue material, it is tremendous to discriminate particular cell types present in the tissue using preferentially specific surface marker(s), to be able to isolate the cells in a live state. When isolating human epithelial cancer cells, it is crucial to discriminate them from endothelial cells, blood cells and mainly from stromal fibroblasts.

In order to identify useful marker for fibroblast detection, we tested three commercially available antibodies recognizing human fibroblasts surface markers (anti-fibroblasts, anti-fibroblasts activation protein FAP, anti-fibroblast surface protein FSP). We confirmed expression of these markers to a different degree on *in vitro* cultivated human foreskin fibroblasts (HFF-1) cell line. Surprisingly we found that not only fibroblast, but also different non-cancer or cancer-derived epithelial human cell lines cultivated *in vitro* may express different levels of these markers.

Since acquisition of mesenchymal phenotype by epithelial cancer cells is associated with the process of epithelial-mesenchymal transition (EMT), we tested whether EMT phenotype may be associated with expression of fibroblast surface markers in epithelial cells. Indeed, our results confirmed that some of these tested fibroblast surface markers were upregulated in response to EMT induction. Finally, we aimed to confirm our results also using human tumor tissue samples.

In summary, our results imply two important facts. First, different epithelial-derived *in vitro* cultivated cell lines with mesenchymal phenotype may express surface markers commonly used for fibroblasts detection. Secondly, using these makers as a marker for negative selection of fibroblasts during isolation of epithelial cancer cells may lead to unwanted elimination of epithelial cells undergoing EMT.

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76 Auxin and cytokinin interactions during de novo organogenesis in Arabidopsis thaliana

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Plant cells are able to undergo a postembryonic *de novo* organogenesis, thus regenerate plant organs from previously differentiated cells. The long known phenomenon of *in vitro* plant regeneration suggests the principal role of two phytohormones, cytokinin and auxin. The root or shoot identity of newly formed organs was shown to be dependent on concentration ratios of these two phytohormones.

To determine the role of cytokinin/auxin interplay in *de novo* organogenesis, we used hypocotyl explants system. We performed deep morphological analysis of primordia structure in WT, transgenic lines with depleted endogenous cytokinins (*Pro35S:AtCKX2* and *Pro35S:AtCKX3*) and in cytokinin receptors' mutants (*ahk*) during the first five days of cultivation. Generally, the highest number of root primordia was observed on medium with auxin only, while increasing cytokinin concentration led to decreasing number of primordia and disorganization of their structure. The higher resistance to the increasing concentration of exogenous cytokinin was visible in *Pro35S:AtCKX* lines followed by *ahk* mutant lines. Measurement of cytokinin content reflected changes in endogenous cytokinin levels during organogenesis. Our results also suggest the role of cytokinins in controlling primordia identity.

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77 Fluorinated ethylene propylene: promotion of keratinocyte proliferation by argon plasma treatment

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Fluorinated ethylene propylene (FEP) is a thermostable and biologically inert polymer which can be favourable for biomedical and research applications, however, its surface hydrophobicity strongly limits cell adhesion. In order to increase FEP surface wettability, and thus enhance cell adhesion, we modified its surface by argon plasma. Advantageously, this facile and reproducible method for material surface modification does not influence its bulk mechanical properties. We modified FEP matrices by Ar plasma with the power of 3 and 8 W for 20, 40, 80, 120, 160, and 240 s. Then we used fluorescence microscopy and image analysis to study adhesion, morphology and proliferation of human keratinocytes of HaCaT cell line cultivated on those matrices. On pristine FEP, the cell adhesion and proliferation was negligible. In contrast, cell proliferation on FEP matrices modified with 3 W Ar plasma was, almost regardless of the Ar plasma exposition duration, comparable to control standard tissue culture polystyrene (PS). A more detailed view on cell adhesion and intercellular connections was obtained by scanning electron microscopy analysis. To further evaluate the overall effects of modifications of FEP matrices on cell metabolic activity, we performed a spectrophotometry-based WST-1 test. The metabolic activity of cells growing on modified FEP matrices was considerably increased compared to pristine FEP and approached that of cells on PS. Therefore, the Ar plasma treatment may be a useful tool to positively influence the biocompatibility of FEP.

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78 Materials and technologies for an in vitro model of the cardiac tissue

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The aim of this work is firstly to overcome the current lack of models for the study of the physiological conditions through the development of an in vitro dynamic model, able to reproduce the myocardial tissue.

To satisfy the target an elastomeric biodegradable polyurethane was synthesized through a two steps procedure starting from poly-ε-caprolactone diol (Mn = 2000 Da), 1,4-butane diisocyanate and L-lysine ethyl ester. Then this polyurethane was processed via Thermally Induced Phase Separation (TIPS) under application of a cooling gradient in order to obtain oriented fibers texture, like the cardiac muscle tissues topography. Polyurethane scaffolds obtained with TIPS were functionalized with fibronectin, one of the main components of the cardiac ECM proteins. The functionalization was performed by plasma treatment with acrylic acid, followed by the activation of carboxylic groups by N'-(3-dimethylaminopropyl)-N- ethylcarbodiimide in combination with N-hydroxysuccinimide, and the coupling with fibronectin. The constructs were characterized by SEM, contact angle, XPS and mechanical test, showing mechanical properties and morphology similar to those of native tissue (Fig.1). Functionalized scaffolds were seeded with cardiac cells prepared from neonatal Sprague-Dawley rats. To evaluate the cardiomyocyte self-beating activity, scaffold were analyzed from four days after cell seeding every two days, by using fluorescent microscopy. Time lapse analysis demonstrated that cells beat synchronously from day 7 and for over 50 days.

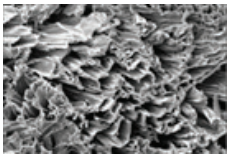

Fig. 1
SEM micrograph of the scaffold

References

[1] Sartori S, Boffito M, Serafini P, Caporale A, Silvestri A, et al 2013 Reactive and Functional Polymers 73, 1366 – 1376.

79 Controling cell behavior by eliciting specific cell-biomimetic surface interactions

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The adjustment biomimetic surface properties of the material through immobilization of extracellular matrix derived peptide motifs are important to facilitate effective interactions between cell receptors and the surface of artificial scaffolds. These biomimetic surfaces should suppress the protein adsorption from the biological media to which they are exposed to, cancel the non-specific cell-material interactions and, at the same time, elicit a specific cell response to biologically active surface immobilized molecules.[1] Herein we present a detailed physic-chemical study of dense PEO brushes prepared by end-tethering of hetero-bifunctional PEOs (M_n=2,000–20,000) to polydopamine (PDA)-modified surfaces from a reactive melt.[2,3] The presence of alkyne distal end-group on the PEO chains was used to couple azidopentanoic-GGGRGDSGGGY peptide utilizing a copper-catalyzed Huisgen azide-alkyne „click" cycloaddition reaction (CuAAC). The peptide surface concentration was tuned from complete saturation of the PEO surface with peptides (1.7×10^5 fmol·cm^{-2}) to values still relevant for cell adhesion studies (<1.0×10^3 fmol·cm^{-2}). The performance of the prepared surfaces is demonstrated on cell cultures of endothelial cells.

References

[1]. V. Proks et al., Macromolecular Bioscience 12, 1232-1242, 2012.
[2] O. Pop-Georgievski O. et al., Langmuir 28, 14273-14283, 2012.

80 Nanostructured surfaces for studying the cellular interactions

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Cell-cell and cell-extracellular matrix interactions play crucial role in biological processes such as cell adhesion, proliferation and differentiation. To study cellular response, the microenvironment is usually mimicked by random immobilization of bioactive molecules on cultivation surfaces *in vitro* or by their distribution directed just in micrometer orders. The main goal of this work was to develop the technique for patterning of biomolecules on substrates at the nanometer level, which corresponds to organization of receptors on the cell membranes. For this purpose, we used recent state-of-the-art lithographic techniques for preparation with highest resolution, focused ion and focused electron beam-induced depositions (FIBID, FEBID) of metal ions to glass substrates. Subsequently, we invented also the dehalogenase-based system for binding bioactive molecules.

Characterization of nanostructures using atomic force microscopy revealed resolution of 100 nm reached by FIBID and sub-50 nm by FEBID. This system has promising potential for the future studies of cellular interactions involved for wide array of detailed biological analysis and applications.

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81 Natural polysaccharide Gum Karaya as novel biomaterial in regenerative medicine

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Natural polysaccharides are biodegradable, freely available, relatively cheap and nontoxic materials with great potential in medical applications. The polysaccharide hydrogels supports wound healing by providing moist environment and can be utilized as wound dressing for soft tissue regeneration (e.g. skin burns or ulcers).

One suitable candidate is Gum Karaya (GK), anionic polysaccharide containing β-D-galactose, L-rhamnose, β-D-glucuronic acid and D-galacturonic acid. Natural GK is partially acetylated high molecular weight polymer (Mw ≈ 9 mil Da), water insoluble, having high swelling and retention capacity, high viscosity and inherent antimicrobial activity. [1, 2, 3]

The objective of this study is to prepare a soluble sample of GK by alkali treatment followed by designing new procedure for GK hydrogel preparation which meets requirements for use as wound healing coverings. Prepared samples were characterized by SEM, FTIR, NMR, TGA, DSC and rheology.

References

[1] B. Singh, N. Sharma: Development of novel hydrogels by functionalization of sterculia gum for use in antiulcer drug delivery. Carbohydrate Polymers. 2008, 74, p. 489-497.
[2] B. Singh, L. Pal: Development of sterculia gum based wound dressings for use in drug delivery. European Polymer Journal. 2008, 44, p. 3222-3230.
[3] D. Verbeken, S. Dierckx, K. Dewettinck: Exudate gums: occurrence, production, and applications. Applied Microbiology and Biotechnology. 2003, 63, p. 10-21.

82 Surface activation of wood by plasma treatment

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Among the biomaterials, the wood is by far the most widely used. It is 3D biocomposite which is light, structurally stiff, naturally bio-degradable, regrowable and economic. All these properties make it absolutely unique among all the construction materials. Still, its properties can be further enhanced and so today, many wood products are actually engineered wood. Some tropical hardwoods exhibit very good resistance to pests and humidity thanks to the natural oils in their tissue. However, it complicates the gluing necessary for production of the engineered wood.

Plasma surface treatment is one of the most significant areas of plasma applications. During such treatment, active species of plasma react only with the surface of material, leaving the bulk material unchanged. Doing so may significantly influence many properties of surfaces, such as wetting or adhesion.

Our experiments [1] deal with plasma surface treatment of various wood species in order to enhance their hydrophilic properties. As a plasma source we used atmospheric pressure microwave plasma jet working in argon, but exposed to ambient atmosphere. The changes on wood surface were evaluated using water uptake time and contact angle measurement. The results showed that microwave plasma treatment was able to achieve significant improvement of wood wettability even after treatment time in matter of seconds. In the best cases, as much as 100x shorter water uptake time was achieved after only one second of plasma treatment.

References

[1] L. Potočňáková, J. Hnilica, V. Kudrle, *International Journal of Adhesion and Adhesives* 45, 2013.

83 Role of pores in preparation of transparent zirconia nanoceramics

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Zirconia has become a popular bioceramic that can be used in implants, dental posts, abutments and fixed partial dentures (overlaid with veneering porcelain). Recently, the increased number of clinical failures of zirconia implants was observed . The main reasons for these failures are build-up stresses during material preparation, and low temperature degradation where zirconia yields to martenzitic type transformation. To avoid these problems, the zirconia tends to be manufactured with smaller grains, preferably nanocrystals, in order to obtain a higher strength, and better resistence to low temperature degradation. Moreover, the nanocrystalline grains can provide the zirconia optical transparency, which is good for aesthetic reasons. To reach such goal, the mastering of every step of the preparation is necessary. In our case, we introduce two type of the zirconia powders, where one of them possess hierarchical pore structure. With understanding and controlling of pore closure during sintering process, we were able to prepare the transparent zirconia with grain size of 87nm, in-line transmittance of 67.5% and no low temperature degradation.

84 TiO_2 nanostructured surfaces for biomedical applications

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Functionalization of titanium surface becomes one of the important biomedical applications since 40% of present medical implants are based on titanium or titanium alloys.[1,2] This work presents nanofabrication of titanium dioxide nanostructured surfaces prepared by anodic oxidation in organic and acidic electrolytes. Morphological structure of anodized TiO_2 was modified by changing the preparation conditions such as anodization time, applied voltage, temperature, Ti layer thickness and electrolyte composition (e.g. content of fluoride, water or organic additives) viscosity, conductivity, and pH. Two approaches were used for the fabrication, one-step and two-step anodization of titanium layer or titanium/aluminium bilayer. Prepared TiO_2 surfaces were characterized by scanning electron microscopy (SEM). SEM images has shown the nanorods and mushroom like nanostructures prepared via one-step anodization of titanium/aluminium bilayer, nanoporous or nanotubular character of one-step anodized titania and nanoporous/nanotubular nanostructures via two-step anodized titania. These TiO_2 nanostructured surfaces will be used for biomedical application such as quantification of cell adhesion and proliferation due to its excellent biocompatibility, high chemical stability, and low toxicity. [1]

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References

[1] ROY, P. et al. TiO_2 Nanotubes: Synthesis and Applications. *Angewandte Chemie International Edition*, 2011, vol. 50, no. 13, pp. 2904-2939. ISSN 1521-3773.
[2] HUO, K. et al. Fabrication, modification, and biomedical applications of anodized TiO_2 nanotube arrays. *Rsc Advances*, 2014, vol. 4, no. 33, pp. 17300-17324.

85 3D Cell Culture of Mouse Lung Stem/Progenitor Cells

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Homeostasis and regeneration of adult tissues is sustained by resident stem cells that co-ordinately proliferate and differentiate to produce progeny for replacement of old or damaged cells. In adult lung epithelium, different populations of lung stem and progenitor cells (LSPCs) have been identified. They reside in different anatomical regions throughout the respiratory tree and can give rise to multiple epithelial lineages of proximal and/or distal airways of the lung. However, our understanding of cellular hierarchy in adult lung and microenvironmental signals that regulate LSPC self-renewal and differentiation remains incomplete. To properly address these questions in vitro, development of physiologically relevant 3D models has been necessary.

We developed a protocol for isolation of LSPC that takes advantage of the unique abilities of stem cells to survive in non-adherent conditions and to self-renew. In this assay, LSPCs form spheroids (lungospheres) of several distinct phenotypes, which most likely correspond to distinct parental LSPC types. Lungospheres can be serially passaged and their proliferation, self-renewal and differentiation is regulated by FGF signalling. When embedded into 3D Matrigel, lungospheres proliferate and form large cystic or branched structures in response to FGF signalling.

Our lungosphere and 3D cell culture assays provide useful tools to assess stem/progenitor properties of distinct lung epithelial cell populations and to study lung epithelial-stromal interactions in vitro.

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86 Interactions of stem cells with conductive polyaniline

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Polyanilines (PANIs) are conductive polymers, which can respond to various stimuli by changes in their optical, electrical, chemical and mechanical properties. Moreover, PANIs have an advantageous physical properties like high electrical conductivity, excellent electronic and optical properties, good redox and ion-exchange activity and environmental stability. Additionally, their synthesis is relatively cheap as well as different PANIs derivatives can be obtained from common chemicals. All of these facts taken together point to an unmistakable conclusion: PANIs are an attractive compounds for bioengineering and biomedical studies.

In our work we aim to adopt several types of PANIs to stem cells proliferation and differentiation. In this pilot studies we have shown that tested compounds are not cytotoxic and can be used for the tissue cultures. Especially, functionality of electro-sensitive cells like cardiomyocytes and neural cells might be improved, possibly because of PANIs conductivity. It would be valuable for potential cell replacement and regeneration therapies. These are the reasons why we are interested to answer the question about usefulness of PANIs applications in various studies.

87 TiO_2 nanotubes and nanowires doped by Ag nanoclusters – fabrication of antibacterial coatings for dental implants

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Among biocompatible materials, which are used in the production of dental implants, titanium and titanium alloys are known. Formation of TiO_2 or hydroxyapatite coatings on them, is used to enhance bone growth, as such surfaces would mimic the extracellular matrix with which cells normally interact.
In our research we used Ti6Al4V alloy (Titanium Grade 5) and we created on their surface coatings of titania nanotubes (TNT), obtained by anodic oxidation and titania nanowires (TNW) synthesized during thermal oxidation. These coatings were doped by silver spherical nanoclusters of defined diameters, in order to add antimicrobial properties to TiO_2 nanocoatings. This defined size of silver nanoclusters is important from biological point of view, but also it is important to obtain silver grains, which do not coat totally the existing TiO_2 film. The dopping process were carried out according known procedure, with the use of atomic layer deposition method (ALD). [Ag(fod)(PEt_3)], where fod - 2,2-dimethyl-6,6,7,7,8,8,8-heptafluorooctane-3,5-dionato ligand, has been used as silver precursor and depositions were made on PEALD reactor – Beneq TFS 200 [1]. The used growth temperature was 120°C. The only parameter, which was changed during the deposition series, was the number of deposition cycles: 50, 100, 150 and 200. Obtained results show that it is possible to tailor the diameter size of silver nanoclusters using ALD method and that it is possible to optimize the parameters of deposition process to obtain silver nanograins on 3D substrats, for example on dental implants.

References
[1] Maarit Kariniemi, Jaakko Niinisto, Timo Hatanpää, Marianna Kemell, Timo Sajavaara, Mikko Ritala, Markku Leskelä, *Chem. Mater*. 2011, 23, 2901–2907

88 Human adult liver progenitor cells – a novel model for in vitro hepatotoxicity assessment

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The occurrence of toxic compounds in the environment presents potential risk for both environment and human health. Although *in vitro* models are widely used in toxicity testing, their relevance and predictive value is limited by abnormalities of commonly used cancer-derived continuous cell lines, by inconsistency and instability of primary cell cultures, as well as by inadequate representativeness of *in vivo* microenvironment in the traditional 2D cell cultures. Adult stem cells derived from intact tissue provide an alternative that can more accurately predict toxicity. To develop a novel tool for effective liver toxicity screening, we introduced protocols using normal (HL1-1) and hTERT-immortalized (HL1-hT1) human hepatic stem cell lines. These cell lines are characterized by expression of stem and liver oval cell markers (Oct-4, α-fetoprotein, vimentin), high proliferation potential, ability of anchorage-independent growth, and lack of gap junctional intercellular communication (GJIC). Ability of these cells to differentiate into hepatocyte-like cells allows to study effects of toxicants on different cell types and potential oncogenic events. Cell models have successfully been used in both traditional 2D cell cultures, as well as in more relevant 3D cultures using our optimized protocol based on micromolded agarose microplate arrays. We demonstrate that adult human liver stem cells provide a relevant *in vitro* model for the identification of new chemical hazards or studies of chemopreventive agents, and for further characterization of their mechanisms of action.

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89 Characterization and cell compatibility of polyaniline films with different stabilizers

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Polyaniline is conducting polymer with broad variety of preparation and modification methods which can significantly enhance its applicability in different applications field. Its application in biotechnology or biomedicine, e.g. in biosensors are in the center of attention of scientific community. The use of four stabilizers (PVP; SDS; Tween and F108) within two preparation conditions (H2O or HCL VK). The surface characteristics, electrical properties and cell compatibility were determined using contact angle measurement, van-der Pauw method and mouse fibroblasts, respectively. The results show that used stabilizer significantly impact not only the surface and electrical properties but also the cell compatibility. From the mentioned stabilizer and preparation conditions, the SDS_HCl seems to preferentially combine both a conductivity and cell compatibility.

90 Aberrant TGFβ superfamily signalling maintains mesenchymal phenotype of breast cancer cells

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Plasticity of cancer cells, characterised by dynamic and reversible transition between different phenotypes of cancer cells, is a very common cause of malignant cancer progression. EMT (epithelial-to-mesenchymal transition) and MET (mesenchymal-to-epithelial transition) are two of the most occuring phenotype changes in adenocarcinomas *in vivo*. These events together with clonal selection are driven by various factors, e.g. immune system and microenvironment, and are responsible for metastasis formation. Unfolding the mechanisms of cancer cell plasticity might contribute to future therapeutical approaches. Breast cancer stem cells (BCSC) are suggested to be drivers of metastasis, resistance to chemotherapy and radiation. BCSC were previously shown to co-exist in diverse mesenchymal and epithelial states in different tumor sites. To understand the plasticity in BCSC we used HER2/neu-overexpressing primary mouse mammary carcinoma cell line and its relapsed HER2/neu antigen-negative variants (ANV). FACS-based screen using 252 validated antibodies against selected surface antigens identified distinct interplay between cell phenotype and expression of molecules responsible for interaction with extracellular matrix. Precise analysis of TGFβ superfamily pathway revealed stabilised expression of Inhibitor of differentiation 1 (ID1) as a consequence of aberrant signalling in mesenchymal cancer cells, but not in their epithelial counterparts. We also observed different expression of surface stem cell markers, transcription factors in general responsible for stemness, EMT regulators and mediators of metastasis amongst cell lines. In summary we showed that regulation of stemness and cancer-associated plasticity is tightly related and under context-dependent control of TGFβ superfamily signalling pathway.

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91 Anisotropic Crystalline Protein Nanolayers as Multi-Functional Biointerface for Patterned Co-Cultures of Adherent and Non-Adherent Cells in Microfluidic Devices

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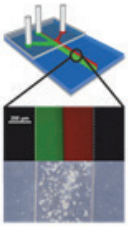
The spatial arrangement of cells in their microenvironment is known to significantly influence cellular behavior, thus making the control of cellular organization an important parameter of in vitro co-culture models. However, recent advances in micropatterning co-culture methods within biochips do not address the simultaneous cultivation of anchorage-dependent and non-adherent cells. To address this methodological gap we combine S-layer technology with microfluidics to pattern co-cultures to study the cell-to-cell and cell-to-surface interactions under physiologically relevant conditions. We exploit the unique self-assembly properties of SbpA and SbsB S-layers to create an anisotropic protein nanobiointerface on-chip with spatially-defined cytophilic (adhesive) and cytophobic (repulsive) properties. While microfluidics control physical parameters such as shear force and flow velocities, our anisotropic protein nanobiointerface regulates the biological aspects of the co-culture method including biocompatibility, biostability, and affinity to nonadherent cells. The reliability and reproducibility of our microfluidic co-culture strategy based on laminar flow patterned protein nanolayers is envisioned to advance in vitro models for biomedical research.

References

[40] M Rothbauer, P Ertl, BA Theiler, M Schlager, UB Sleytr, S Küpcü. Anisotropic Crystalline Protein Nanolayers as Multi-Functional Biointerface for Patterned Co-Cultures of Adherent and Non-Adherent Cells in Microfluidic Devices. Advanced Materials Interfaces, 2014, DOI: 10.1002/admi.201400309..



92 X-ray microscopy utilizing world largest photon counting detector

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Imaging of low attenuating material is very challenging using conventional X-ray imaging techniques. Nowadays photon counting detectors with large sensitivity for low photon energies provides adequate solution. A modular dual-source tomography scanner equipped with unique word largest area photon counting detector WidePIX was employed for 2D radiography scanning. Multiaxial scanner has large geometric variability; it allows scanning milimeter scale objects as well as flat objects of the area up to ~1 m^2. Along with the variability in magnification and tube/detector positioning, examined object can be displayed with a micrometric scale resolution even for relatively large specimens. Resultant image tiled from number of high resolution radiograms can easily reach tens or hundreds of megapixels. Inspection of the biological material scanning will be presented.

References

[1] Llopart, X., Ballabriga, R., Campbell, M., Tlustos, L., Wong, W. Timepix, a 65k programmable pixel readout chip for arrival time, energy and/or photon counting measurements. (2007) *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*, 581(1-2), pp. 485-494. DOI: 10.1016/j.nima.2007.08.079. ISSN 01689002.
[2] Jakubek, J., Jakubek, M., Platkevic, M., Soukup, P., Turecek, D., Sykora, V., Vavrik, D. Large area pixel detector WIDEPIX with full area sensitivity composed of 100 Timepix assemblies with edgeless sensors. (2014) *Journal of Instrumentation*, **9**(04), pp. C04018-C04018. DOI: 10.1088/1748- 0221/9/04/c04018.
[3] Turecek, D., Holy, Jakubek, J., Pospisil, S., Vykydal, Z. Pixelman: a multi- platform data acquisition and processing software package for Medipix2, Timepix and Medipix3 detectors. (2011) *Journal of Instrumentation*, 6(01), pp. C01046-C01046. DOI: 10.1088/1748-0221/6/01/C01046. ISSN 1748-0221.

93 N^6-adenosyl methylation regulates vascular development and hormonal response in Arabidopsis

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Despite recent stunning progress, the physiological role of N6-adenosyl methylation (m6A) of mRNA and the identity of associated molecular machinery remain practically unknown. It is also very little known about its role in pattern formation, in plants, as well as in other eukaryotes. Protein AHP6, a member of signaling cascade of a plant hormone cytokinin, plays a critical role during protoxylem development and its expression relies also on other phytohormone, auxin. We isolated mutant *emb2016-6*, which shows a reduced *AHP6prom:GFP* activity, accompanied with aberrant protoxylem formation, resistance to auxin 2,4-D and to ethylene. Consistently, RNA sequencing revealed that emb2016-6 shows altered expression of numerous genes associated with regulation of auxin dependent processes, and also specific changes in its transcriptome.

emb2016-6 codes for a weak allele of an embryonically lethal and evolutionarily conserved gene, which was proposed to function in mRNA processing. We used tandem affinity purification and a reciprocal cross-linking interactomics approach to find proteins interacting with EMB2016. We co-purified proteins previously known to be involved methylation in mRNA, as well as another uncharacterized conserved RING finger protein. We demonstrate that depleting of m6A levels leads to phenotypic defects similar to those seen on *emb2016-6*. We also found that depletion of EMB2016 expression in Arabidopsis and its homolog in HeLa cells leads to a strong reduction of m6A levels in mRNA.

94 High-resolution label-free 3-D imaging of live cells' organelles from bright-field transmission nanographs

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Current effort in biological and medical research is aimed at obtaining a detailed spatiotemporal map of a live cell interior. This can be fulfilled using label-free bright-field light-transmitted imaging.

In the contribution, we will introduce a 3-D segmentation algorithm of object spread functions of organelles and organelles themselves from a bright-field transmission microscopic z-stack of an unlabelled live cell. The principle of the algorithm is based on searching for pixels of unchanged intensities in the course of cell spread function. Divergence derived from the Rényi entropy has been used for modelling the shapes of the living cell´s organelles and their PSFs.

The mathematical approach enables to differentiate objects of the size of one voxel (few thousands of cubic nanometres, i.e., a box side of tens of nanometres) and describe the spectral properties and dynamics of the cell interior.

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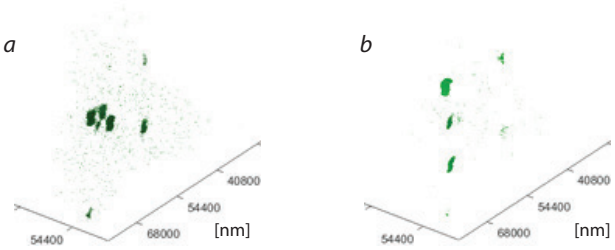

***Interior of a MG63 cell in G-channel**. Light-diffracting and absorbing objects including negative interferences (a), autofluorescent objects and positive interferences (b).*

95 Determination of biocompatibility of materials for organic electronic biosensors

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Use of electronic biosensors based on organic materials attracts a great deal of attention since it can lead to effective, more sensitive, cheap and thus more competitive technology. The level biocompatibility of organic materials is an essential feature for their application in this regard. A thin layer of selected materials (e.g. PEDOT:PSS, TIPS/pentacene, etc.) was deposited into a culture plate. The stability of materials was analyzed in a physiological aqueous environment. Next the biocompatibility assays were carried out with NIH 3T3 fibroblasts. These assays included a test of toxicity and determination of cell adhesion. Materials showed different degree of stability in aqueous environment ranging from excellent water resistance (e.g. parylene) to swelling (some PEDOT:PSS formulations). Similarly the ability to support living cell adhesion and growth varied largely among different materials. Cell viability assays mostly reflected the stability of materials in aqueous environment and their support for adhesion of living cells.

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96 Telomerase associated proteins characterization

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Telomerase is an enzyme which lenghtens telomeres. They are positioned at the end of linear eukaryotic chromosomes. Enzyme telomerase is studied for several decades and has been characterized in many organisms. The composition of telomerase differs in between organisms. But each telomerase has conserved areas which occur in all organisms. Viability of cell depends on functionality of telomerase. If the mechanism of telomeric lenghtening is performed, the cell is alive. If the function of telomerase is interrupted, telomeres are shortened and cell is managed to senescence. Protection of telomeres is not performed only by telomerase. Important part that protects telomeres are proteins or protein complexes interacting with telomeric DNA. For example telomeres of mammals contain shelterin complex which is responsible for protection of telomeres.

For now no structural and functional equivalent of shelterin has been found in plants and equally not all interacting partners of telomerase has been found. So one of goal was to find out some plant proteins interacting with telomerase. By tandem affinity purification (TAP) a lot of proteins interacting with telomerase were obtained. The main task was to check protein-protein interactions between newly found proteins and catalytic subunit of telomerase called TERT. But it could be good idea to check protein-protein interactions between this newly found proteins and known proteins in which interactions with telomeres or TERT was proved. Interactions can be found by three methods called: Bimolecular Fluorescence Complementation (BiFC), Immunoprecipitation (IP) and Yeast two Hybrid system (Y2H).

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97 Segmentation and 3D imaging of cartilage tissues in mouse embryo head

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Understanding face developmental processes requires accurate 3D visualisation of the entire embryo head. This study is realised on the cartilage structures in the head at the end of the embryonic stage of mice. X-ray computed microtomography (microCT) [1] has the potential to produce high resolution quantitative 3D imaging of such a small biological sample. Even with the best staining procedure which produces differential soft tissue contrast, the cartilage has a low detectability in the tomographic image and the segmentation has to be done manually.

We present the automatic segmentation method of the cartilage tissue of olfactory system in microCT data. This method is based on the 3D region growing method [2] and dynamic thresholding criteria. The quality of an input data is important for a reliability of the segmentation method. For this purpose the Canny's edge detector [3], which belongs to more advanced detectors and less sensitive to noise, is used. The closing (morphological) operation was applied on the segmented region to remove small holes inside of the region. As the region growing algorithm generate big errors on the large volume data, the algorithm was applied on the partial data volume.

The segmentation procedure is demonstrated on 14 day and 16 day mouse embryo. The samples were stained by phosphotungstic acid (PTA) [4] and embedded in agarose gel. The CT measurement were conducted using an industrial system GE phoenix v|tome|x L 240 equipped with a 180 kV/20W maximum power X-ray nanofocus tube. 3D models were created from the segmented region and compared with the reference model done by manual segmentation. The proposed segmentation marked almost entire the cartilage structure but the operator intervention is still needed especially at the end of the structure where the cartilage boundary is unclear. However, the automatic approach saves a significant processing time.

References

[1] HSIEH, J. Computed tomography : principles, design, artifacts, and recent advances, Wiley, 2009
[2] WALEK, P., LAMOŠ, M., JAN, J. Analýza biomedicínských obrazů,
ISBN 978-80-214-4792-9, 2013.
[3] JAN, J. Medical Image Processing, Reconstruction and Restoration: Concepts and Methods, CRC, Press, 2005. ISBN 0-8247-5849-8.
[4] B. D. Metscher, MicroCT for Developmental Biology: A Versatile Tool for High-Contrast 3D Imaging at Histological Resolutions, Developmental Dynamics 238 (2009) 632-640.

Acknowledgments: This work was supported by the project "CEITEC – Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068) from European Regional. Development Fund. J.K. acknowledges the support of Brno University of Technology on the frame of grant FSI-S-11-22 (Application of advanced optical methods).

98 Calcium partially stabilized ZrO_2 ceramics nanocrystals for bioaplications

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Zirconia is well known ceramic material being used in dental and hip joint replacement surgery. It is mainly due to its bioinert properties and transformation toughening mechanisms acting in their microstructure, which can impart the components made out of them very interesting mechanical properties. The Y2O3 partially stabilized zirconia (Y-PSZ), together with Ce-ZrO_2 and Mg-ZrO_2, is the most typical and often studied material for many decades. But nowadays, thanks to the new technologies, technics and available materials, we can substitute Y^{3+} ions by biogenous Ca^{2+} ions and we can achieve more biologically active material while maintaining similar mechanical properties.

The aim of this study is to prepare a nanocrystalline partially CaO-stabilized ZrO_2 (Ca-PSZ) using the the sol-gel method. Then to find the proper molar concentration of Ca^{2+} ions and temperature of solid state reaction for the highest content of tetragonal phase in Ca-PSZ system. In the end to prove its bio-properties by *in vitro* tests.

The percentual amount of crystalline phases (monoclinic, cubic, and tetragonal) in Ca-ZrO_2 materials was observe using the XRD analysis. The amount of phases was changing by varying the calcium molar concentration (2.5, 5, 11, 17, 18.5 and 50 $\%_{mol}$) and solid-state reaction temperature (550 – 1200 °C). Morphology and the chemical composition of the product were studied by the SEM/EDX analysis and the surface area was determined by the BET analysis. Cytotoxicity of materials was tested *in vitro* using direct contact assay method. From the achieved results it was determined that the best sample for another tests and research is sample made out of 5 %mol Ca^{2+} and solid-state reacted at temperature of 550 °C because of its nanostructure.

99 Intraspinal aplication of human neural precursors improve progression of ALS

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Amyotrophic lateral sclerosis (ALS) is characterized by progressive degeneration of motor neurons in the cortex, brainstem and spinal cord. This disease is manifested paralysis and death within an average of 3 to 5 years from disease onset. Although the mechanisms responsible for motor neuron degeneration in ALS remain unclear, cell transplantation is considered a promising approach for replacing damaged cells and promoting neuroprotective and neuroregenerative repair.

We investigated the effects of intraspinal implantation of human neural precursors (NP-iPS) derived from a clone of human iPSCs (IMR90) in the SOD1-transgenic rat model of ALS. NP-iPS were transplanted into asyptomatic rats (7 weeks old) and symptomatic rats (25 weeks old). The course of the disease and functional recovery after cell transplantation was monitored behaviourally (BBB, rotarod, gript stregth tests). Spinal cords were collected to evaluate the effect of transplanted NP-iPS on endogenous regenerative processes and fate of the graft (histological evaluation). Quantitative polymerase chain reaction (qPCR) was used to evaluate expression growth factors and apoptosis-related genes.
We found that NP-iPS ameliorated disease progression, significantly improved motor activity and prolonged survival. The cells survived in the spinal cord till the end-stage of the disease and normalized the expression of studied host genes (NGF, IGF-1 and BDNF) and stabilized the expression of apoptosis-related genes (BAX, BCL-2 and Casp-3). These results suggest NP-iPS a promising for future cell-based therapy of ALS.

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100 Extraordinary deformation capacity of smallest carbohelicene springs

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The extraordinary deformation and loading capacity of nine different [ ]carbohelicene springs under uniaxial tension up to their fracture were computed using the density functional theory. The simulations comprised either the experimentally synthetized springs of hexagonal rings or the hypothetical ones that contained irregularities (defects) as, for example, pentagons replacing the hexagons. The results revealed that the presence of such defects can significantly improve mechanical properties. The maximum reversible strain varied from 78% to 222%, the maximum tensile force varied in the range of 5 nN to 7 nN and, moreover, the replacement of hexagonal rings by pentagons or heptagons significantly changed the location of double bonds in the helicenes. The fracture analysis revealed two different fracture mechanisms that could be related to the configurations of double and single bonds located at the internal atomic chain. Simulations performed with and without van der Waals interactions between intramolecular atoms showed that these interactions played an important role only in the first deformation stage.

101 EFFECTS OF OPIOID PEPTIDES ON CARDIOMYOGENESIS IN VITRO

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Endogenous opioid peptides such as dynorphins and enkephalins play an important role in regulation of many biological processes including heart development and cardiovascular function. They are ligands of opioid receptors localized on the outer cellular membrane as well as the inner nuclear membrane. Interestingly, some data suggest that dynorphins and enkephalins could potentiate heart regeneration through stimulation of the cardiac stem cells regenerative potential. However, the effects of opioid peptides on cardiomyogenesis at cellular and molecular level are not well understood. Therefore, the aim is to clarify the effects of dynorphin A and dynorphin B during cardiomyogenesis *in vitro*.

The action of selected peptides was tested using the model of differentiation of mouse embryonic stem cells (cell line R1) into cardiomyocytes *in vitro* via the formation of embryoid bodies. The expression of markers characterizing cardiomyogenesis and the expression of the opioid receptors were determined by quantitative RT-PCR, by Western blot and by flow cytometry.

Results confirmed functionality of the employed model showing increased expressions of gene specific for cardiac phenotype (Nkx2.5, α-actinin, myosin heavy chains α and β) and decreased expressions of gene characteristic for the pluripotent phenotype of the cells (Oct4, Nanog). Nevertheless, supplementation of the differentiating cells by the opioid peptides did not show any statistically significant effects on the course of cardiomyogenesis. It was despite the fact that the gene expressions of opioid receptors were increased during cardiomyogenesis. However, preliminary flow cytometric analysis suggest that the location is especially on the nuclear membrane.

In conclusion, these primary data did not confirm significant effects of dynorphins during cardiomyogenesis *in vitro*, probably due to the low occurrence of opioid receptors on the cell surface membrane.

102 MULTIPARAMETRIC ANALYSIS OF COMPLEX CELLULAR RESPONSE TO THE EXPERIMENTAL TREATMENT USING FLOW CYTOMETRY

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Flow cytometry is convenient method that enables analysis of cellular phenotype on single cell level and therefore confirms its irreplaceability not only in clinical routine but also in basic research.

Regular analysis of cellular response to the experimental anti-cancer treatments in vitro usually includes detection of variety of parameters e.g. viability, apoptosis, cell cycle distribution, DNA synthesis and DNA damage. These parameters are usually analysed in parallel using different methods. Multicolour flow cytometry brings the possibility to perform multiparametric analyses and establishes protocols for simultaneous detections of different parameters. However, combination of immunophenotyping and analysis of complex cellular response in the term of detection of different intracellular markers is still challenging.

Here, we report a protocol for simultaneous flow cytometric detection of viability, CD surface antigens, DNA synthesis and quantification, detection of DNA damage and apoptosis. We performed this analysis on representative panel of cell lines derived from human and mouse prostate cancer, colon cancer and B-lymphoma.
The approach resulted in a seven-parameter assay that could complement the routine cell-based tests and bring more information to the analysis of complex cellular response in phenotypically characterized cell populations.

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103 Replication and transcription machineries are efficiently separated in nucleoli

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In mammalian cells, ribosomal genes coding for the 18S, 5.8S and 28S rRNAs are transcribed within the nucleolar structures called FC/DFC units (i.e. Fibrillar Centers with adjacent Dense Fibrillar Components). Since these genes are intensively transcribed throughout interphase, some kind of mutual adjustment is needed to avoid collision of the DNA polymerase and RNA polymerase machineries. In this work, we measured correlation of various replication and transcription signals in the nucleoli of HeLa, HT-1080 and NIH 3T3 cells employing a specially devised software for analysis of confocal images. Using a stable cell line expressing GFP-RPA43 (subunit of RNA polymerase I) and RFP-PCNA (the sliding clamp protein) based on HT-1080 cells, we found that replication and transcription signals are more efficiently separated in nucleoli than in the nucleoplasm. Analysis of single molecule localization microscopy (SMLM) images indicated that transcriptionally active FC/DFC units did not incorporate DNA nucleotides. Moreover, comparing distribution of distances between the transcription (FU) and replication (EdU) signals on the one side and between fibrillarin (which is a structural component of DFC) and EdU signals on the other, shows that EdU signals tends to localize closer to fibrillarin than FU signals. Our data indicate that FC/DFC units may provide a structural basis for the efficient separation of replication and transcription in the nucleoli.

Reference
[1] E. Smirnov et al, Separation of replication and transcription domains in nucleoli, Journal of Structural Biology, 2014. 188 (3): 259-266.

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104 Optimization of liquid jet system for laser-induced breakdown spectroscopy analysis

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KEY WORDS: liquids, heavy metals, synchronization, optimization, double pulse LIBS

Laser-induced breakdown spectroscopy (LIBS) enables direct in-situ analysis of samples in any state of matter1. This capability of LIBS enables its utilization in vast variety of applications, for instance bioapplications2 and online monitoring. We report on the optimization of a system for direct elemental analysis of samples in liquid phase using LIBS technique. This system consists of a peristaltic pump and a thin specially designed nozzle producing a thin flow of liquid solution/suspension. Such arrangement was used to reduce splashes of liquid and sedimentation of suspension and thus to improve the repeatability of an experiment. Firstly, stepping frequency of the peristalti c pump was synchronized with a flashlamp of ablation laser source. Using such synchronization, changes in pressure and hence volume of liquid along the step of the peristaltic pump were mitigated. Changes in the liquid flow volume affect the laser ablation process in the sense of so-called effective volume function3. Other phenomenon affecting LIBS signal fluctuations, moving breakdown, was also studied. Afterwards, single pulse (SP; 1064 nm Nd:YAG laser pulse) and double pulse (DP; 1064 nm and 532 nm Nd:YAG laser pulses) LIBSsystems were optimized to obtain best possible signal-to-noise ratio. The performance of SP and DPLIBS in a detection of traces of heavy metals was estimated. As a result, significant improvement in sensitivity (limits of detection) of DP LIBS system for analysis of Cu and Pb was observed.

References

[1] Miziolek, A.W., V. Palleschi, and I. Schechter. Laser Induced Breakdown Spectroscopy. *Cambridge: Cambridge University Press*, 2006. ISBN 978-051-1541-261.
[2] Pořízka, P., *et al. Sensors* **14** (2014) 17725-17752. DOI: 10.3390/s140917725.
[3] Yaroshchyk, P. *et al. Applied Spectroscopy* **58** (2004) 1353-1359. DOI: 10.1366/0003702042475592.

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105 Centrosomal abnormalities and multipolar divisions in human embryonic stem cells

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Human embryonic stem cells (hESC) are pluripotent cells that have potential for possible use in clinical medicine as the source of differentiated cells for cell replacement therapy and tissue engineering. However one of the major obstacle is that hESC are genomically unstable and acquire many karyotypic abnormalities. It was previously described that many hESC lines contain a significant population with supernumerary centrosomes during mitotic division [1]. Aim of this study is to examine how hESC containing supernumerary centrosomes divide. For in vitro observation we created transgenic cell lines carrying a histone H2A-tagged with green fluorescent protein (GFP). We acquired multiple time lapse movies during 72 hours and analysed all mitotic divisions. We counted more than 20000 mitoses and detected 3 % of multipolar divisions. Majority of multipolar divisions were tripolar, giving rise to three cells. We also evaluated the length of cell cycle between bipolar and tripolar types of division and found that median time required for bipolar division is 18 hours, however for tripolar division is much longer, around 29 hours. Interestingly majority of daughter cells from multipolar mitoses were viable and some of them divide further by bipolar or multipolar fashion. Our data shows that multipolar mitoses in undifferentiated hESC give rise to viable and dividing cells. Therefore we propose that the inability of hESC to arrest multipolar divisions is the mechanism leading to karyotypic abnormalities.

References

[1] Z. Holubcová, Human embryonic stem cells suffer from centrosomal amplification, Stem Cells, 2011, ISSN 1066-5099.

106 Nanoparticle-Enhanced Laser-Induced Breakdown Spectroscopy of Metallic Samples

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KEY WORDS: signal enhancement; plasma properties, nanoparticles

Laser-Induced Breakdown Spectroscopy (LIBS) has been widely used in modern analytical chemistry because it offers a series of advantages such as fast response, applicability to any type of sample, practically no sample preparation, remote sensing capability and speed of analysis [1]. From an analytical point of view and in comparison with other analytical methods (ICP and LA-ICP based techniques) a moderate disadvantage of this technique is its quantitative analysis. Thus improvement of LOD (Limit of detection) is one of the most important issues. Recent and the least explored method for this improvement is utilization of nanoparticles for the increase in the analytical sensitivity. A number of already published studies do not still satisfactorily describe the dynamics and processes responsible for this enhancement. Innovative concepts in this field reflect pioneering papers of De Giacomo [2,3].

Metallic samples may content many additives in different concentrations (trace elements) and their low concentrations are often under LOD of typical LIBS systems. Optically active nanoparticles may be utilized in order to improve the sensitivity of LIBS analysis of metallic samples without any need for changing the classical set-up but manipulating with the sample preparation only. Application of NPs at the sample surface is simply possible by dropping of their suspension on the sample surface. The nanoparticles are used also in different fields, in biology mostly as biomarkers for different molecules to study their concentration in different tissues, as for example biocompatible nanoparticles used for in vivo tumor targeting [4]. We see the potential of LIBS method in mapping of nanoparticles in tissues in order to utilize the advantages of this method for fast analysis.

In this study selected nanoparticles (gold and silver) in distinct sizes and in different solutions (10 µl) were applied on metallic standards. After drying they were analyzed with by the means of LIBS in spatially resolved maps. These maps clearly demonstrate that intensity of trace elements is improved in the region of a droplet containing NPs. Those results suggest viable utilization of nanoparticles in biology as well as in industry.

References

[1] A. W. Miziolek, V. Palleschi and I. Schechter, *Laser Induced Breakdown Spectroscopy*, (Cambridge University Press, 2006).
[2] A. De Giacomo, R. Gaudiuso, C. Koral, M. Dell'Aglio and O. De Pascale, Analytical chemistry **85** (2013) 10180-10187. [3] A. De Giacomo, R. Gaudiuso, C. Koral, M. Dell'Aglio and O. De Pascale, Spectrochimica Acta Part B: Atomic Spectroscopy **98** (2014) 19-27.
[4] X. Qian, X.-H. Peng, D. O. Ansari, Q. Yin-Goen, G. Z. Chen, D.M. Shin, L. Yang, A. N. Young, M. D. Wang and S.Nie,
Nature Biotechnology **26** (2008) 83-90.

107 Influence of EPD rate and applied electric field on surface roughness of Ca-phosphate biomaterials

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Bioceramic materials based on Ca-phosphates were prepared by electrophoretic deposition (EPD). The main objective of this work was study of dependence of the deposition rate and applied voltage influenced by chemical composition of dispersions on the roughness of bioceramic surfaces. Deposition rate and electric field were affected by electric conductivity of dispersions which was controlled by concentration of monochloroacetic acid (MCAA) and lithium chloride (LiCl) in dispersions. Dispersions were composed from 0.00, 4.25, 12.15 and 21.25 wt.% MCAA, 15 wt.% of ceramic load and 2 propanol medium. The electric conductivity of the dispersion was further modified by adding an indifferent electrolyte (LiCl) in an amount of 0, 0.10, and 0.25 g/L into the dispersion. High content of MCAA and LiCl in dispersion caused an increase of electric conductivity of the dispersion. High electric conductivity led to the decrease of electric field strength and to the deceleration of the deposition rate. In this work was shown that the dispersion with low electric conductivity, high deposition rate and applied electric field formed significantly rough relief on the deposits surfaces. Optimal conditions for the formation of relatively smooth surfaces was achieved by high electric conductivity, low deposition rate and applied electric field.

108 Large Area Scanning Thermal Microscopy

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Large area Scanning Probe Microscopy (SPM) [1,2] is a technique bridging the gap between high resolution surface properties mapping 3D state-of-the-art techniques available at limited spatial range in various commercial SPM systems and conventional methods acting on much larger spatial scales like profilometry or confocal microscopy. Development of 3D tools capable of acquiring high resolution data on large areas is very important not only for metrological support in semiconductor or optical industry, but it is also crucial for traceability of the SPM techniques itself. In this contribution, we present a design of a combined large area Scanning Thermal Microscopy (SThM) and Infrared Radiometry system, which was developed for analysis of local temperature and local thermal conductivity variations with very high spatial resolution. Main purpose of this device is to form a traceability route from conventional thermal conductivity and temperature measurement methods (which have poor spatial resolution, but excellent temperature/conductivity resolution) towards Scanning Thermal Microscopy, which has high spatial 3D resolution, but is still understood more like qualitative rather than quantitative technique. Measurement examples using unique device prototype are shown on both passive and active experimental setups. Limiting factors of the measurement uncertainty on different samples are discussed, too.

References

[1] Klapetek, P., et al. „Large area high-speed metrology SPM system." *Nanotechnology* 26.6 (2015): 065501.
[2] Dai, Gaoliang, et al. „Metrological large range scanning probe microscope." *Review of Scientific Instruments* 75.4 (2004): 962-969.

109 PPARgamma ligand supports anticancer effects of oxaliplatin on colon and prostate cancer cells

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Epidemiological studies indicate a possible connection between development of colon and prostate cancers, and metabolic syndrome, obesity, increased intake of lipids or cholesterol. The peroxisome proliferator-activated receptors (PPARs) are transcription factors that are important modulators of lipid and sugar metabolism, inflammation and cell proliferation. We hypothesized that combination of PPARgamma ligand – rosiglitazone and DNA damaging drug - oxaliplatin might increase their respective antiproliferative effects on colon (HT-29) and prostate (BPH-1 CAFTD03) cancer cells. Our results showed that rosiglitazone increased activity of PPARgamma receptor, and in combination with oxaliplatin, it significantly decreased proliferation of both colon and prostate cancer cells. Because tumours often become resistant to conventional chemotherapy, we prepared oxaliplatin-resistant colon cancer cell lines. Our results showed that combination of PPARgamma ligand and oxaliplatin decreased cell proliferation rate and blocked cell cycle progression in G2/M phase also in the resistant cells. Moreover, this combination almost completely abolished colony-forming capacity of both parental and resistant colon cancer cells. We further identified important differences of specific protein expression between cancer cell lines and clinical samples from cancer patients. Immunohistochemistry staining showed that in samples from prostate cancer patients, higher levels of PPARgamma are present in prostate cancer samples than in benign prostate hyperplasia. Using cancer cell lines established from patient with prostate cancer we confirmed results obtained with permanent prostate cancer cell lines. These experiments verified that combination of PPARgamma ligand and oxaliplatin decreased proliferation and increased cell death of epithelial prostate cancer cells.

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110 BIOCOMPATIBILITY ASSESSMENT OF CYCLOPROPYLAMINE PLASMA POLYMERS STUDIED BY COHERENCE-CONTROLLED HOLOGRAPHIC MICROSCOPY

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KEY WORDS: quantitative phase imaging, Coherence-Controlled Holographic Microscope, cyclopropylamine, amine-rich coating.

The understanding of the surface-cell interaction plays important role for the biomaterials development and bioengineering. We present the Coherence-Controlled Holographic Microscope (CCHM) built in Brno University of Technology as a tool for a reliable assessment of the biocompatibility of the amine-rich coatings [1].
Although it is already known that amine groups increase the cell adhesion and proliferation, the influence of the amine layers properties on cell adhesion need to be further investigated. Imaging and assessment of surface-cell interaction is an arduous task, since the cells are weakly scattering and absorbing specimens. The CCHM enables to acquire speckle-free optically-sectioned quantitative phase images of live cells in high contrast without using any labels [2]. The phase image contains quantitative information, from which valuable parameters directly related to the cell mass can be obtained. Based on those parameters, the biocompatibility of the surfaces is evaluated.
The stable amine-rich coatings were prepared by low pressure plasma polymerization of cyclopropylamine using radio frequency capacitively coupled discharge. The normal human dermal fibroblasts were plated on sterilized plasma treated coverslips and cultivated for 2 days. The cell-surface interaction was imaged by the Coherence-Controlled Holographic Microscope and biocompatibility was assessed. Results show that amine-rich films proved to act as biocompatible surfaces that enhanced cells adhesion and proliferation.

References

[1] P. Kolman, and R. Chmelík, „Coherence-controlled holographic microscope," *Optics Express* 18.21, 21990-22003 (2010).
[2] T. Slaby et al., „Off-axis setup taking full advantage of incoherent illumination in coherence-controlled holographic microscope," *Optics Express* 21.12, 14747-14762 (2013).

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111 Preparation of matrices from Antheraea pernyi silk and its characterization

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Silk is a natural protein-based biopolymer produced by many species of insect and spiders. The main component of the insect silk fiber is fibroin, a protein with remarkable, highly repetitive structure. Fibroin itself is composed of both hydrophilic (N- and C-terminus) and hydrophobic (repetitive and non-repetitive) domains[1]. Periodical organization of these parts enables a formation of final fiber. It was shown, that owing to high biocompatibility, biodegradability, temperature stability and remarkable mechanical properties, silk-based materials have a great potential for biomedical applications[2].

Our project has been focused on silk of wild silkmoth *Antheraea pernyi (A. pernyi)*. Our goal was to prepare and characterize matrices from A. pernyi silk fibroin (APSF), which would be suitable for use in regenerative medicine research.

Silk from this wild silkmoth is, in comparison to widely-used *Bombyx mori* silk, very hard to dissolve. Nevertheless, we successfully developed a new method for *A. pernyi* fibroin solubilization directly from the silk gland. This new method allows us to isolate full length fibroin of high purity. From the APSF solution were prepared 2-D matrices. During the matrix preparation, a fixation procedure for stabilization of required beta sheet structures of fibroin is essential. Scanning electron microscopy and Atomic force microscopy analysis enabled us to study the surface characteristic of our novel material, which are strongly dependent on the fixation conditions. The effect of pH and ionic strength during the fixation was studied in a detail. Also other methods confirmed the possibility to design APSF matrices with different stability and hydrophobicity suggesting us APSF as a new type of promising biomaterial applicable for next research.

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References

[1] Gosline, J., Guerette, P., Ortlepp, C., and Savage, K.: Comp. Biochem. Phys. A 124, 34 (1999).
[2] Vepari, C., Kaplan, D. L.: Prog. Polym. Sci. 32, 991–1007 (2007).

112 Porous hydrogels as 3D cell scaffolds mimic the anatomy of bone marrow extracellular matrix

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One of the most basic components of biological experiments are cell cultures on plates and dishes. It is generally accepted that a three dimensional (3D) culture of cells rather than a two dimensional (2D) culture is very essential for adequate development of tissue. Culturing cells in 3D models can lead to superior cell viability, differentiation and function compared to existing conventional culture systems.

We prepared 3D scaffolds from porous hydrogels poly(2-hydroxyethyl methacrylate) (pHEMA), poly(2-hydroxyethyl methacrylate-co-2-aminoethyl methacrylate) p(HEMA-co-AEMA) and p(HEMA-co-AEMA) modified with frequently used cell adhesion peptide Arg-Gly-Asp (RGD). Hydrogel scaffolds were manufactured with four different pore sizes (125 µm, 200 µm, 300 µm, 350-450 µm). Our aim was the scaffold structure to be geometrically similar to the 3D morphology of supporting bone marrow tissue in a trabecular bone. The 3D scaffold was also designed to conform to biocompatibility, sufficiently large surface area for cell attachment, high porosity for cell migration, proliferation and transport of nutrients and substantial transparency for inspection of scaffold with optical techniques. The prepared scaffolds demonstrated the convenient system for the investigation of cell-cell and cell-matrix interactions. We tested human bone marrow HS-5 cell line, human embryonic kidney HEK 293 cell line and primary human B-cells chronic lymphocytic leukemia (B-CLL cells).

In conclusion, we have demonstrated that p(HEMA-co-AEMA) hydrogel scaffold modified with RGD peptide with pore diameter 350-450 µm can be used for the three dimensional cell culture. RGD-conjugated p(HEMA-co-AEMA) hydrogel can promote cell adhesion, spreading and proliferation. Even we got evidence about behavior of primary human B-CLL cells in co-cultivation with human stromal cells HS-5 in the 3D p(HEMA-co-AEMA) hydrogel scaffolds with RGD peptide which mimic the anatomical and physical features of bone marrow.

113 Novel Non-toxic Bactericidal Electrospun Gelatin/Oxycellulose Nanofibers for Medical Applications

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The present work deals with a preparation of (bio) polymer nanofibers produced by Nanospider technology. The nanofibers are biocompatible, bio-adhesive, and porous mimicking the structure of the extracellular matrix of tissues. Gelatin nanofibers modified by the addition of sodium or calcium salts of oxidized cellulose (oxycellulose) were successfully prepared. Gelatin is biocompatible, biodegradable and has a high absorption capacity. Oxycellulose and its salts are biocompatible, biodegradable and exhibit both hemostatic and bactericidal effects. The crosslinking process of nanofibers was another important step that improved hydrolytical stability and enhanced mechanical properties of obtained nanofibers. Significant bactericidal efficiency of prepared nanofibers was tested via chemical bioluminescence utilizing *Escherichia coli* bacteria strain. Moreover, the nanofibers were seeded with NCI-H441 cell line (human lung papillary adenocarcinoma cells) proving good adhesion and proliferation on the nanofibers surface that can serve e.g. as the lung-mimicking tissue for the *in-vitro* treating lung cancer. Therefore, novel non-toxic bactericidal gelatin/oxycellulose nanofibers represent a promising option for application in surgery and regenerative medicine, particularly in soft tissue engineering.

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114 Comparison of Particle Size Distributions Measured Using Different Techniques

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Particle size distributions were measured by means of various techniques, including multisample analytical centrifuge (LUMiSizer), scanning electron microscopy (JEOL JSM-7600F), and photon cross-correlation spectroscopy (SYMPATEC NANOPHOX). These techniques are based on different measuring principles; evaluation of transmission profiles recorded during separation process (analytical centrifuge), a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens (scanning electron microscopy), and 3D cross-correlation of scattered light intensities (photon cross-correlation spectroscopy). The comparison of particle size distributions was performed for spherical polystyrene particles and nonspherical silica flakes. It is shown that particle shape strongly affects the results obtained by different techniques. For spherical polystyrene particles, the particle size distributions obtained by analytical centrifuge, scanning electron microscopy, and photon cross-correlation spectroscopy agree well. Moreover, analytical centrifuge provides a powerful tool for the caharacterization and quality control of dispersions. Worse results among different particle measurement techniques were obtained in case of nonspherical particles. The particle size distribution of the sample determined by analytical centrifuge is in good agreement with that obtained from photon cros-correlation spectroscopy. Scanning electron microscopy yielded a mean diameter that is about 20% lower than those obtained from analytical centrifuge or photon cros-correlation spectroscopy. This fact is probably due to the shrinkage of particles during sample drying and high-vacuum measurements.

115 Hematopoietic developmental potential is variable in pluripotent stem cell lines

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The clinical application of hematopoietic precursors generated from human pluripotent stem cells (hPSCs) is still limited by low efficient differentiation protocols and functional defects in the derived cells. Moreover, the potential of various hPSCs to differentiate into blood cells has not been examined properly. In this study, protocols for hematopoietic differentiation were applied to available human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC) lines to determine their hematopoietic developmental potential. These protocols included different methods for hPSC cultivation and embryoid bodies (EBs) formation and composition of differentiation media. The efficiency of hematopoietic differentiation was found to be variable among hPSC lines. No protocol optimization enabled generation of $CD34^+$ hematopoietic precursors from available hESC lines CCTL-12 and CCTL-14. On the contrary, these precursors were detected during hiPSC differentiation in basic media supplemented with three cytokines. The yield of precursors was variable, ranging from 0.8 to 10.3 percent of $CD34^+$ cells and from 1.0 to 10.6 percent of $CD43^+$ cells at day seven of differentiation. This yield was further increased when hiPSCs were differentiated for ten days in media with richer cytokine supplement. Nevertheless the variability among hiPSC lines was retained, 6.4 to 16.3 percent of cells were $CD34^+$ and 7.8 to 20.0 percent of cells were $CD43^+235^+$ precursors of primitive hematopoiesis, while $CD45^+$ precursors of definitive hematopoiesis comprised only from 0.4 to 4.7 percent. These results indicate that hematopoietic developmental potential of individual hPSC lines is characterised by significant variability that has to be overcome before blood cells derived from hPSC could be used for clinical applications.

116 Biosilica-loaded poly(ε-caprolactone) nanofibers mats: A morphogenetically active surface scaffold for the mineralization of the osteoclast-related SaOS-2 cells

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Bioprinting/3D cell printing procedures for the preparation of scaffolds/implants have the potential to revolutionize regenerative medicine. Besides biocompatibility and biodegradability, the hardness of the scaffold material is of critical importance to allow sufficient mechanical protection and, to the same extent, allow migration, cell-cell, and cell-substrate contact formation of the matrix-embedded cells. In the present study, we present a strategy to encase a bioprinted, cell-containing, and soft scaffold with an electrospun mat. The electrospun poly(ε-caprolactone) (PCL) nanofibers mats, containing tetraethyl orthosilicate (TEOS), were subsequently incubated with silicatein. Silicatein synthesizes polymeric biosilica by polycondensation of ortho-silicate that is formed from prehydrolyzed TEOS. Biosilica provides a morphogenetically active matrix for the growth and mineralization of osteoblast-related SaOS-2 cells in vitro. Analysis of the microstructure of the 300-700 nm thick PCL/TEOS nanofibers, incubated with silicatein and prehydrolyzed TEOS, displayed biosilica deposits on the mats formed by the nanofibers. We conclude and propose that electrospun PCL nanofibers mats, coated with biosilica, may represent a morphogenetically active and protective cover for bioprinted cell/tissue-like units with a suitable mechanical stability, even if the cells are embedded in a softer matrix.

References

[41] Müller WEG, et al., Biotechnol. J., 2014, 9: 1312-1321
[42] X.H. Wang, et al., Soft Matter, 2012, 8: 9501
[43] X.H. Wang, et al., Curr. Opin. Biotechnol., 2012, 23: 570
[44] W.E.G. Müller, et al., Acta Biomater., 2011, 7: 2661
[45] W.E.G. Müller, et al., Chem. Eur. J., 2013, 19: 5790

117 The study of biocompatibility of magnetic particles using amplification methods

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Magnetic particles for biotechnology applications usually consist of a number of components: magnetic cores are covered with a variety of functionalized low-or high-molecular compounds (polymer layer). Iron oxides and some substances used to stabilize magnetic cores can reduce biocompatibility of the proposed particles; therefore perfectly encapsulated cores are very important for their application. The real-time polymerase chain reaction (qPCR) is very sensitive for the presence a number of chemicals. The increasing inhibitors concentration decreases the amplification efficiency and thus the reaction curve slope decreases.

The experiments for the estimation magnetic nano- and microparticles inhibitory effect or biocompatibility respectively on the course of real-time PCR were proposed. The set of magnetic non-porous microspheres covered by different content of carboxyl groups, PEGylated monodisperse magnetic poly(2-hydroxyethyl methacrylate) and poly(glycidyl methacrylate) microspheres, newly synthetized types of particles - lanthanide-doped up converting nanoparticles -$NaYF_4$:Yb^{3+}/Er^{3+} particles and compounds used for magnetic core encapsulation were studied. Linear regression analyses were used for the evaluation of the influence of the particles or compounds on the qPCR course.

It was confirmed that the qPCR can be used for the study of compatibility of magnetic particles with DNA amplification. Real-time PCR methods based on shift of *Cq* values after addition of different amounts of the particles to DNA was found as a suitable method for evaluation of their inhibition effect.

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118 Advanced Materials for Implants Made with Laser Additive Technologies

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The fabrication of medical implants requires use of biocompatible materials and innovative additive technologies, mainly based on laser sintering. The laser technologies work in non-equilibrium physical and chemical metallurgical conditions and produces end-use complex parts. The metallic material in powder form is sintered layer by layer according to CAD-data of 3D model [1]. The material characteristics and processing conditions result in wide scale of microstructural and mechanical properties of the materials and 3D designs. This is the main advantage for a production of advanced implants used in surgery and orthopaedics. The 3D model of customized implant can be acquired by exploitation of Computed Tomography (CT) scan or Magnetic Resonance Image (MRI) [2] and directly fabricated by additive technology. Other medical potential of additive technology is possibility to use biocompatible materials like titanium alloy Ti-6Al-4V and chromium-cobalt alloys Co-Cr-Mo, Co-Cr-W-Ni, Co-Ni-Cr-Mo, Co- Cr-Mo. This study investigates influence of variation of process parameters (laser power, scan speed, thickness of powder layer) on final quality of part and evaluates potential of additive technologies in medical applications. Two materials were used for fabrication of samples – the austenitic stainless steel 1.4404 (AISI/SAE 316L) and Ti-6Al-4V (α+β) alloy. The stainless steel which shows a good acid resistance (chemical industry, apparatus engineering) was chosen for its deformation hardening, high strength and no need of protective atmosphere while fabrication. Mechanical properties of samples were evaluated by hardness, compression and tensile tests and quality of 3D surfaces was assessed with Alicona IF G4 [3]. Fabrication of Ti-6Al-4V samples, where argon-protective atmosphere continues, and their testing will follow in next steps of the study.

References

[1] K. Boivie, R. Karlsen, C. Van Der Eijk, Material Issues of the Metal Printing Process. NTNU, Trondheim, 2011.
[2] L. Loeber, S. Biamino, U. Ackelid, S. Sabbadini et al., Comparison of selective laser and electron beam melted titanium, in solid freeform fabrication proceedings. Austin: University of Texas, 2011. pp. 547-556, ISSN 1053-2153.
[3] P. Trubačová, Modern method of implant productions by Rapid prototyping: Bachelor work. Brno: Brno University of Technology, 2014. 51 pp.

119 Biofeedback and Modern Imaging Methods for Rehabilitation

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Nowadays, assessment of functional changes of human musculoskeletal system is based primarily on subjective evaluation of physiotherapists, orthopaedist and rehabilitation specialists. Expert community lacks appropriate objective methods for quantification of functional changes such as muscular imbalance or uncoordinated posture of a body segment. Specialists also lack a possibility to objectively assess effects of targeted rehabilitation. Since it is impossible to objectively evaluate positive changes of conservative therapy, arthroscopic surgery is oftentimes performed, irreversibly disrupting surrounding tissues. Besides the direct invasive intervention in the integrity of the body segment, there are other disadvantages of surgical intervention: the surgery itself is expensive and time demanding, and convalescence of patients is long.

Thus our goal is creation of new possibilities of non-invasive diagnostics of ankle-joint and in-depth assessment of functional posture of extremities in static posture, potentially in dynamic posture too.

Basic requirement is establishment of external referential points on the lower extremity. Mutual spatial arrangement of these points will provide predictive value about the position of internal structure. Such referential points will be recorded by modern imaging sensors. One of the possible suitable sensors is Kinect One from Microsoft. Contrary to common colour cameras, Kinect One allows in-depth 3D sensing of the scene which enables high quality reconstruction of the scanned part of the patient's body, especially more faithful kinematic bone model. Compared to other typically used optical proximity sensors, Kinect One is cheaper while providing sufficient parameters important for the given application (space as well as length resolution, framerate, latency, etc.).

120 A simple microviscometric approach based on Brownian motion tracking

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Viscosity – an integral property of a liquid – is traditionally determined by mechanical instruments. The most pronounced disadvantage of such an approach is the requirement of a large sample volume, which poses a serious obstacle, particularly in biology and biophysics when working with limited samples. Scaling down the required volume by means of microviscometry based on tracking the Brownian motion of particles can provide a reasonable alternative. We report a simple microviscometric approach which can be conducted with common laboratory equipment. The core of this approach consists in a freely available standalone script to process particle trajectory data based on a Newtonian model. In our study, this setup allowed the sample to be scaled down to 10 µl. The utility of the approach was demonstrated using model solutions of glycerine, hyaluronate, and mouse blood plasma. Therefore, this microviscometric approach based on a newly-developed freely-available script can be suggested for determination of the viscosity of small biological samples (e.g. body fluids).

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121 LA-12 mediates effective stimulation of TRAIL-induced apoptosis in human prostate cancer cell lines and patient-derived tumor samples

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Our research is focused on investigation of the cytotoxic and cytostatic properties of platinum(IV) adamantylamine ligand-containing complex LA-12, an interesting drug with promising anticancer potential. Previously, we and others demonstrated LA-12 as highly effective in killing of several cancer cell types including those resistant to cisplatin or oxaliplatin. We newly showed a potent ability of LA-12 to enhance TRAIL (tumor necrosis factor-related apoptosis inducing ligand)-induced apoptosis in several human prostate cancer cell lines, regardless of their p53 status. Importantly, we also demonstrated a significant stimulation of apoptosis induced by the combination of LA-12 and TRAIL in prostate cancer cells from tumor biopsy specimens obtained from human patients suffering from this disease. We also suggested the molecular mechanisms behind these cytotoxic cooperative effects, especially at the level of mitochondria. These results will be presented and discussed within our contribution.

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122 Evolution of the NSE1-NSE3-NSE4 subcomplex of the human SMC5/6 complex

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The SMC5/6 complex is crucial for maintenance of genome stability through its functions in chromosome segregation, DNA repair and replication fork stabilization, and is essential in most organisms. It consists of two arms, SMC5 and SMC6, which are interconnected through hinge domains on one side and through the NSE1-NSE3-NSE4 subcomplex on the other side, forming a ring-shaped structure.

In most organisms, there is only one variant of NSE3 and NSE4 proteins. However, in placental mammals NSE3 evolved into a large family of MAGE proteins and NSE4 evolved into a family of several NSE4/EID proteins. The NSE1 has a RING-finger domain, which is typical for E3 ubiquitin ligases. Previously, we showed that interactions of the MAGE proteins with their NSE4/EID partners are evolutionarily conserved. Here, we are focusing on MAGE – RING-finger proteins interaction analysis.

We employed the yeast two-hybrid system to screen a human RING-finger protein library against several MAGE baits. We identified a number of potential MAGE-RING interactions and confirmed several of them in co-immunoprecipitation experiments. We examined these MAGE-RING pair interactions in detail and focused on MAGEA1-TRIM31 pair which resembles the Nse3-Nse1 interaction pattern. We showed that both MAGEA1 and TRIM31 bind to NSE4 directly, forming a TRIM31-MAGEA1-NSE4 complex reminiscent of the NSE1-NSE3-NSE4 trimer. In addition, MAGEA1 interaction stimulates TRIM31 ubiquitin-ligase activity, resembling the NSE3-driven stimulation of NSE1. These results are suggesting that MAGEA1 functions as a co-factor of TRIM31 ubiquitin-ligase and that the TRIM31-MAGEA1-NSE4 complex may have evolved from an ancestral NSE1-NSE3-NSE4 complex.

References

[1] Kozakova L, Vondrova L, Stejskal K, Charalabous P, Kolesar P, Lehmann AR, Uldrijan S, Sanderson CM, Zdrahal Z, Palecek JJ.: The Melanoma-associated Antigen 1 (MAGEA1) Protein Stimulates the E3 Ubiquitin-ligase Activity of TRIM31 within a TRIM31-MAGEA1-NSE4 Complex. (2015) Cell Cycle Mar 19;14(6):920-30

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123 Syntesis of non-fouling poly(HPMA) brushes by photoindused SET-LRP

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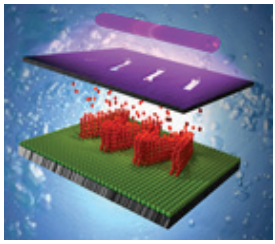
Non-fouling polymer brushes with complex architecture are fundamental for improving the performance of biomaterials that interface with biological media or tissues, as well as to confer specific function to surfaces.

In this work, polymer brushes of [*N*-(2-hydroxypropyl methacrylamide (HPMA) were grafted from silicon wafers by photoinduced single-electron transfer living radical polymerization. Despite the low concentration of copper catalyst (80 ppb), excellent control of the polymerization was achieved for the first time for the grafting of methacrylamide monomer by a Cu-catalyzed polymerization. The living nature of the polymerization was clearly demonstrated by formation of diblock copolymer brushes and re-initiation experiments. By exploiting the lightinduced characteristic well-defined micropatterns were grafted from the surface. Finally, negligible fouling was observed after contact with undiluted blood plasma.



References

[1] M. Vorobii, A. de los Santos Pereira, O. Pop-Georgievski, N. Yu. Kostina, C. Rodriguez-Emmenegger, V. Percec, *Polym. Chem*., 2015,6, 4210
[2] J. Laun, M. Vorobii, A. de los Santos Pereira, O. Pop-Georgievski, V. Trouillet, A. Welle, C. Barner-Kowollik, T. Junkers, *Macromo.Rapid.Commun.* 2015, available online.

124 3D Modeling and Visualization of Patient-Specific Brain Structure and Vessels Based on MR Images

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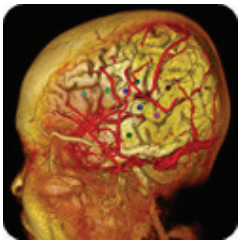
It's important to know the patient specific brain structure and the branch of vessels for the minimally invasive brain surgery. Aiming at depth brain electrode insertion for intractable epilepsy treatment, a complete pre-operative multimodality imaging, image processing and three dimensional visualization procedure was developed. For visualize the brain structure and vessels simultaneously, multi-contrast magnetic resonance images were acquired. The 3D T1 weighted images, and 3D FLAIR images were used for the structure modeling. 3D PCA (phase contrast angiography) [1] was performed, which provides contrast of both cortical veins and arteries in the images. The three image scans were conducted consecutively with the patient's head held still. Based on the multi-contrast image data, the vessels and brain tissues were segmented. A compositely surface and volume rendering procedure was performed for the visualization of the structure. We have accomplished the fusion and visualization of the planned depth electrode trajectories, brain cortex and cortical vessels, and the functional areas of brain (provided by additional fMRI) simultaneously. The 3D modeling and visualization were implemented based on the open source platforms of ITK[2] and VTK[3]. The method has been proven to be an effective way to help the surgeons make surgical plans for locating and removing the epileptic zone.



References
[46] C. L. Dumoulin, Phase contrast MR angiography techniques. Magnetic resonance imaging clinics of North America, 1995, 3(3): 399.
[47] Insight Segmentation and Registration Toolkit (ITK). http://www.itk.org
[48] W. Schroeder, K. Martin, B. Lorensen. The Visualization Toolkit: An Object-Oriented Approach To 3D Graphics, 3rd version. Kitware, 2003

125 Bioactive and biodegradable silica biomaterial for bone regeneration

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Biosilica, a biocompatible, natural inorganic polymer that is formed by an enzymatic, silicatein-mediated reaction in siliceous sponges to build-up their inorganic skeleton, has been shown to be morphogenetically active and to induce mineralization of human osteoblast-like cells (SaOS-2) *in vitro*. We prepared beads (microspheres) by encapsulation of β-tricalcium phosphate [β-TCP], either alone (control) or supplemented with silica or silicatein, into the biodegradable copolymer poly(D,L-lactide-*co*-glycolide) [PLGA]. Experimental studies for those beads revealed no toxicity in the MTT based cell viability assay using SaOS-2 cells. The adherence of SaOS-2 to the surface of silica-containing microspheres was higher than for microspheres, containing only β-TCP. Using these microspheres, first animal experiments with silica/biosilica were performed in female, adult New Zealand White rabbits to study the effect of the inorganic polymer on bone regeneration *in vivo*. The results revealed that tissue/bone sections of silica containing implants and implants, composed of a 1:1 mixture of silica-containing microspheres and silicatein-containing microspheres show an enhanced regeneration of bone tissue around the microspheres, compared to the control implants containing only β-TCP. We propose that based on their morphogenetic activity on bone-forming cells *in vitro* and the results of the animal experiments presented here, silica/biosilica-based scaffolds are promising materials for bone repair/regeneration.

References
[49] X.H. Wang, et al., Soft Matter, 2012, 8: 9501
[50] X.H. Wang, et al., Curr. Opin. Biotechnol., 2012, 23: 570
[51] W.E.G. Müller, et al., Acta Biomater., 2011, 7: 2661
[52] W.E.G. Müller, et al., Chem. Eur. J., 2013, 19: 5790
[53] S.F. Wang, et al., Bone, 2014, 67: 292

126 Biocalcite: A morphogenetic biomaterial in bone regeneration

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Ca-carbonate is the component that builds up the spicules of the calcareous sponges. Recent results revealed that the Ca-carbonate/biocalcite-based spicular skeleton of these animals is formed *via* an enzymatic mechanism. The enzyme that mediates the Ca-carbonate deposition has been identified as a carbonic anhydrase (CA) and has been cloned e.g. from the calcareous sponge species *Sycon raphanus*. Ca-carbonate deposits are also found in vertebrate bones besides the main constituent, hydroxyapatite (HA). By using the human osteogenic SaOS-2 cells it could be shown that after exposure of the cells to Ca-bicarbonate *in vitro*, a significant increase of Ca-deposit formation results. In parallel, the expression of the carbonic anhydrase-II (CA-II) gene becomes upregulated. Finally, it is shown that ortho-phosphate and hydrolysis products of polyphosphate inhibit CA-II activity, suggesting a feedback regulatory system between the CA-driven Ca-carbonate deposition and a subsequent inactivation of this process by ortho-phosphate. We conclude, the hydroxyapatite deposition in bone is preceded by Ca-carbonate precipitation, a process that is driven by an increased CA activity. That is to say, Ca-carbonate crystals act as bioseeds in human bone formation. This discovery may allow the development of novel biomimetic scaffolds for bone tissue engineering. Na-alginate hydrogels, enriched with bicarbonate and biosilica, have recently been demonstrated as a suitable matrix to embed bone forming cells for rapid prototyping bioprinting/3D cell printing applications.

References

[54] W.E.G. Müller, et al., Biomaterials, 2013, 34: 8671
[55] W.E.G. Müller, et al., Acta Biomaterialia, 2014, 10: 450
[56] X.H. Wang, et al., Trends Biotechnol., 2014, 32: 441
[57] X.H. Wang, et al., Beilstein J. Nanotechnol., 2014, 5: 610
[58] X.H. Wang, et al., Calcified Tissue Intern., 2014, 94: 495

127 Getting the knack of woodpecker head knocks

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The question of how woodpeckers avoid cranial and neural damage from the extreme forces they generate by pecking has been studied by morphologists, physicists, and engineers. Some morphological approaches contain analyses only of static forces, while other studies have not included sufficient biological detail. 3D imaging offers an opportunity to improve existing descriptions and models.

One problem is that basic features of the woodpeckers' head anatomy have not been described in sufficient detail. In particular, the exact position of the brain with respect to the skeletal structures and the expected mechanical forces between them have not yet been described adequately. Moreover, more detailed mechanical studies of the impact forces have overlooked one important aspect of avian anatomy, namely cranial kinesis.

We are investigating the skull anatomy of the great spotted woodpecker (*Dendrocopos major*) using x-ray microtomographic (microCT) imaging of whole heads. Using 3D size-calibrated images we could get an exact description of the relations of brain and skeletal elements. Based on these data we are developing a finite-element model that includes both the hard and soft tissue components of the woodpecker head to generate more realistic and biologically relevant results than earlier studies.

128 Contribution and specific effects of ethylene biosynthetic genes on CK- controlled root patterning and development

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The genetic and the molecular basics of plant hormones action and interaction have been extensively studied. One such interaction known for a long time is a cytokinin-ethylene crosstalk. Cytokinins were shown to stimulate production of 1 - aminocyclopropane - 1 - carboxylic acid (ACC), the rate - limiting precursor of ethylene biosynthesis and quickly upregulate three out of four proteins involved in the ethylene biosynthetic pathway. Interestingly, these regulations reveal remarkable tissue and time specificity, taking place predominantly in the root of young Arabidopsis seedlings.

Our current study is focused on the analysis of cytokinin - ethylene interaction in order to identify genes involved in ethylene biosynthesis and affecting cytokinin - controlled root apical meristem (RAM) size and / or cell elongation. For our study we have selected genes involved in ethylene biosynthesis (*AtMS1, AtMAT3, AtMAT4, AtACS5, AtACS9 and AtACO2*) which protein products were found to be regulated by exogenous cytokinin treatments. Our preliminary data shows that when treated for 6 days with 100 nM of aromatic cytokinin 6 – Benzylaminopurine (BAP), the null mutants in some of the aforementioned genes, namely *acs5*, *acs9* and *atms1* were partially resistant to BAP - mediated reduction of root growth. Additionally, some of these mutants have longer RAM when compared to the WT in the absence of exogenous cytokinins. Moreover, the *acs5* mutant line was the most sensitive in terms of the cytokinin-induced shortening of the RAM. Detailed analysis of individual lines and their specific contribution to cytokinin-controlled RAM size and patterning will be presented.

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129 *In vitro* co-culture model to study paracrine signaling between Leydig and Sertoli Cells

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Somatic testicular cells, namely Leydig and Sertoli cells, play a crucial role in testicular steroidogenesis, testicular development and subsequently spermatogenesis. Leydig and Sertoli cells are separated by the blood-testis barrier into two different tissue compartments interacting to each other via paracrine signaling and reciprocally modulating their functions by a release of soluble factors and chemical signals.

In our study, we used mouse Leydig cell line TM3 and mouse Sertoli cell line TM4 and established an *in vitro* co-culture system to study cell responses induced by paracrine signaling between these two cell types. The cells were cultured on Matrigel coating, with the effector cells (Leydig or Sertoli) seeded onto E-Plate Insert and co-cultured with the target cells (Sertoli or Leydig) grown in E-Plate VIEW 96. The growth and other cell responses of the target cells in the presence of different numbers of effector cells were monitored in a real time using non-invasive label-free impedance measurements (RTCA xCelligence SP). We observed that the soluble factors secreted by Sertoli TM4 cells increased proliferation of the target TM3 cells during the exponential phase of growth, whereas Leydig TM3 cells did not have a significant effect on TM4 cells.

The Sertoli/Leydig *in vitro* co-culture model introduced in our lab represents a valuable tool not only for future research of somatic testicular cells functions and interactions, but also to study disruption of paracrine signaling by environmental contaminants and its contribution to the development of male reproductive dysfunctions.

130 Injectable hydrogels prepared from extracellular matrix as scaffolds for spinal cord injury repair

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Spinal cord injury (SCI) often results in permanent neurological deficits as a consequence of the inability of axons to regenerate across the lesion. One possible method of repairing this injury is bridging the SCI lesion with a supportive and stimulatory material.
Extracellular matrix (ECM) derived hydrogels were prepared by decellularization of porcine spinal cord (SC) or porcine urinary bladder (UB) and injected immediately into a spinal cord hemisection cavity. Furthermore, combination of SC-ECM and human umbilical cord Wharton's jelly-derived mesenchymal stem cells (hWJ-MSCs) were studied.

Two weeks after injury both hydrogel types matched well with the surrounding tissue and filled the lesion cavity. Nevertheless, at later time points several cysts developed due to rapid graft degradation. A number of NF-positive fibers and blood vessels grew into the hydrogel treated lesions and no difference was found between SC-ECM and UB-ECM hydrogels at any time points.

The most profound host tissue response to the ECM hydrogels was observed 2 weeks after injury, when downregulation was found in mRNA expression of genes related to inflammation, markers M1 and M2 macrophages and neurogenesis compared to the control SCI lesion. Interestingly, these effects declined or even reversed at the later time points, suggesting that ECM hydrogel degradation plays a significant role in the transient modulation of the innate immune and tissue repair response.

In conclusion, both ECM hydrogels showed significant immunomodulatory and neuroregenerative effects and provided a substrate for bridging tissue after SCI.

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131 Force-assembled $Fe3O_4$ particle chains in polyurethane matrix

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Despite substantial research efforts, the potential of polymer nanocomposites has still not been fully revealed, mainly due to poor control over the dispersion and alignment of nanoparticles (NPs). Since nanocomposite properties are controlled by the structural variables, it is crucial to achieve control over the NP assembly process.
Self-assembly of NPs offers limited control over the NP spatial arrangement. This process results in a poorly controlled variation of simple structures such as agglomerates, clusters and dispersed NPs with the resulting structure strongly dependent a on wide range of thermodynamic parameters.
On the other hand, force-assembly exploits interactions between particles induced by external force fields overcoming the thermodynamic ones. Stimulus of external electric, magnetic or electro- magnetic field is applied as the main force controlling the assembly of NPs. Understanding this process gives us the opportunity to create prescribed NP structures with controlled shape, size, and anisotropy by simple change of the force field. Precise control of structure formation on different length scales (from nano to macro) gives us the opportunity to imitate hierarchical biological structures possessing unique balance of stiffness and toughness. Here, we report on magnetic field force assembly of Fe3O4 nanoparticles in the polyurethane matrix. Resulting NP chain structures were several NP wide and tens of micrometers long aligned along the magnetic force lines. Without the magnetic field, NP agglomerates of random size and shape were formed due to their self-assembly.

References

1. J. Jancar, J.F. Douglas F.W. Starr, S.K. Kumar, P. Cassagnau, A.J. Lesser, S.S. Sternstein a M.J. Buehler. Current issues in research on structure–property relationships in polymer nanocomposites, Polymer. 51 (2010) 3321-3343.
2. P.J. Krommenhoek, J.B. Tracy, Magnetic Field-Directed Self-Assembly of Magnetic Nanoparticle Chains in Bulk Polymers, Particle. 30 (2013) 759-763.
3. U.G.K. Wegst, H. Bai, E. Saiz, A.P. Tomsia, R.O. Ritchie, Bioinspired structural materials, Nature Materials.14 (2014) 23-36.
4. M.A. Meyers, P.Y. Chen, M.I. Lopez, Y. Seki, A.Y.M. Lin. Biological materials: A materials science approach, Journal of the Mechanical Behavior of Biomedical Materials. 4 (2011) 626- 657.
5. J. Aizenberg, J.C. Weaver, M.S. Thanawala, V.C. Sundar, D.E. Morse, P. Fratzl, Skeleton of Euplectella sp: Structural Hierarchy from the Nanoscale to the Macroscale, Science. 309 (2005) 275-278.
6. J.B. Tracy, T.M. Crawford, Magnetic field-directed self-assembly of magnetic nanoparticles. MRS Bulletin. 38 (2013) 915-920.
7 D. Fragouli, R. Buonsanti, G. Bertoni, C. Sangregorio, C. Innocenti, A. Falqui, D. Gatteschi, P.D. Cozzoli, A. Athanassiou, R. Cingolani, Dynamical Formation of Spatially Localized Arrays of Aligned Nanowires in Plastic Films with Magnetic Anisotropy. ACS Nano. 4 (2010) 1873-1878.

132 Role of solvation in viscoelastic properties of hydrogel networks

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The deformation response of hydrogel networks was discussed mostly with respect to the properties of organic networks. However, the solvation of network also plays an important role. In the contribution we describe a solvation of network and its role in the viscoelastic response. The solvation of polyelectrolytes was described by a concept of Bjerrum length (BL). The BL is the separation distance at which the electrostatic interaction is equal to the thermal energy (kT). The BL was calculated from the partial electric charge of solvent and macromolecular network. If the BL is too small, the Van der Waals interactions repulse the water from network and solvation is not observed. In the case that BL is sufficiently large (approximately 0.25 nm and more), the water is attracted by the network and the solvation occurs.

The viscoelastic response of the molecular model is sensitive to the solvation or non-solvation of network. The well solvated model networks are less elastic than the non-solvated ones. The behavior is interpreted as a consequence of three structural effects observed during deformation: change of mixing energy, formation or dissociation of micelles, and deformation of elastic network. By elucidating the relations between solvation of network and structural changes during the network deformation, one may predict hydrogel properties with known electrostatic charge of the network or solvent.

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133 Molecular dynamics simulations for an improved interpretation of 14-3-3ζ FRET measurements

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Förster resonance energy transfer (FRET) is a spectroscopic method widely used to determine inter- and intramolecular distances of biomolecules through distance dependent energy transfer between two fluorophores. Apart from distances, the efficiencies of FRET also significantly depend on the mutual orientation of the fluorophores which are generally not accessible to experiments and often crudely assumed to be isotropic. This assumption might become especially invalid for dyes attached to protein surfaces where special interactions can be formed and cause the dye orientations deviate far from isotropic.

In order to improve interpretation of FRET measurements, overall dynamics of the dyes attached to the protein can be probed by molecular dynamics (MD) simulation. Obtained ensemble averaged values of FRET efficiency can be directly compared to the experimental values.

In this study we simulate selected variants of 14-3-3ζ protein labelled by a set of fluorescent dyes at the N-terminal end. For that purpose we prepared force-field parameters for simulated fluorescent labels compatible with the 54A7 GROMOS force-field. The preliminary results of MD simulations indicate that dyes Alexa Fluor 488 and Alexa Fluor 594 connected to N terminal tail of 14-3-3ζ via flexible oligopeptide linker sample near-isotropic range of orientations.

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134 Effect on cell viability of AgCu nanoparticles

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Ag-Cu binary nanoparticles were synthesized by reduction of Ag and Cu nitrates by sodium borohydride with polyvinylalcohol as a surfaktant. Chemical composition of synthesized Ag-Cu NPs was done using inductively-coupled plasma-optical emission spectrometry (ICP-OES). The optical properties of nanoparticles were monitored using UV-Vis spectrophotometer. The hydrodynamic diameter of nanoparticles was measured by dynamic light scattering (DLS). Zeta potential of Ag-Cu colloids was measured by electrophoretic method. Size and shape of metal core of nanoparticles and morphology of aggregates were investigated by technique of electron microscopy (SEM, TEM and HRTEM). The thermal properties of Ag-Cu nanoparticles were evaluated by differential scanning calorimetry (DSC).

Metallic nanoparticles are widely known as cytotoxicity or antibacterial reagent. The effect of application of metallic Ag-Cu nanoparticles was examined on human ovarian carcinoma cells A2780 with comparison of equivalent concertation of pure PVA. The cell viability was tested by MTT test. Significant decrease of cell viability after the application of Ag-Cu nanoparticles was observed with comparison of the application of pure PVA.

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