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Abstract Book

POLYMER DRUG DELIVERY SYSTEMS FOR ANTI-INFLAMMATORY THERAPY – THEIR IN VIVO BIODISTRIBUTION IN MICE WITH ACUTE ARTHRITIS

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The current therapy of chronic inflammatory diseases (CID), e.g., rheumatoid arthritis (RA), usually only reduces disease symptoms and often leads to severe side effects. The employment of inflammation-targeted nanomedicines may significantly improve CID treatment and lead to complete remission. Among nanomedicines, water-soluble polymer drug conjugates may be highly beneficial in CID treatment due to their passive targeting into inflammation site and the controlled drug release. The polymer conjugates with dexamethasone (Dex) based on hydrophilic, biocompatible and non-immunogenic copolymers of *N*-(2-hydroxypropyl)methacrylamide (HPMA) exhibited superior anti-inflammatory activity in RA murine model compared to free Dex and Dex-loaded liposomes.[1] In order to study the biodistribution of the HPMA-based polymers *in vivo*, several fluorescently labeled polymer systems with different molecular weight were synthesized and their fate in mice with acute arthritis was observed. The HPMA-based polymer carriers were accumulated in inflamed joints to a higher extent than in healthy joints within 48 h when the animals were sacrificed. The accumulation rate was molecular-weight dependent – the carriers with higher molecular weight circulated in blood stream longer resulting in enhanced accumulation rate. Therefore, the HPMA-based polymer carriers enable passive targeting to inflamed tissue and their conjugates with suitable anti-inflammatory drugs may improve CID treatment.

Biography:

Eva Koziolová has completed her PhD from Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic (IMC) in 2016. She was awarded a "Prix de Pharmacie" organized by the French Embassy in Prague in 2016. She passed one-year postdoctoral internship in France at the University of Montpellier and at the University Paris Descartes. She is currently employed at the Department of Biomedicinal Polymers of IMC. Her research is focused on preparation of diverse polymer-based drug delivery systems for effective treatment of cancer and inflammation. She has published 17 papers in reputed international journals and presented her work at numerous international conferences.

References:

1. Quan, L. *et al*., ACS Nano 8 (1), 2014.

LOCAL DELIVERY WITH BIOADHESIVE THERMOSENSITIVE HYDROGELS

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Local delivery is a very interesting strategy for some tumor treatment, such as rectal, intraperitoneal or intra-hepatic tumors. With local delivery, neo-adjuvant and adjuvant effects can be expected, but also distal effect can be achieved which open the way to curative treatments. For these purposes, we conceived bioadhesive thermosensitive hydrogels using mixtures of poloxamers and bioadhesive polymers such as chitosan, xantham gum and alginate. The end points were a temperature of gelification between 15°C and 30°C, an elasticity G' > 10000 Pa at 37°C and an adhesion effect which were measured by rheology. Time of residence was assessed using non-invasive in vivo imaging. A selection of bioadhesive thermosensitive hydrogels was done to be futher evaluated. In vivo, a cocktail of the folfox regimen was entrapped within the hydrogel and evaluated on a rectal model. On a more complex model, including a primary and secondary distal tumor of Colon 26-Luc +, the combination of a surgery by radiofrequency and a local immunostimulation inhibited the growth of distal tumors. The cytotoxic cell response was modulated by an *in situ* injection of a thermo-reversible hydrogel loaded by GM-CSF and BCG, targeting dendritic cells. The efficiency was assessed on evolution of distant microscopic or macroscopic tumors, survival, tumor microenvironment and systemic immunity. These results open a way in the avoidance of local and distal tumor recurrence.

Biography:

Nathalie Mignet has completed a PhD in chemistry on the pro-oligonucleotide approach in 1996. After a post-doc at Lynx Therapeutics, USA and the chemistry department of Sheffield, UK, she started to work on vectorization in the company Capsulis, France. She joined the CNRS in 2000 and she is now the head of the team Vectors for Molecular Imaging and Targeted Therapy. She has published more than 70 papers in reputed international journals. Her research is focused on nanomedicine, imaging and delivery. Since 2014, she is the president of the French Society for Nanomedicine.

NANOSTRUCTURES FOR EFFICIENT IN VIVO RNA INTERFERENCE WITH MULTIPLE POTENTIAL MEDICAL APPLICATIONS

Daniel Scherman, Virginie Escriou, Pascal Bigey, Patrick Arbuthnot

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Because of their unique pharmacodynamics properties allowing the protection of an active therapeutic or imaging agent, its in vivo targeting and its control release, nanoparticles display huge potential for health, both for diagnostic (imaging) and treatment.

RNA interference represents a promising strategy for the treatment of various disorders. Important problems remain to be resolved before clinical practice of this new type of drug, such as that of siRNA stability after injection and SiRNA penetration into target cells. Indeed, the half-life of unmodified siRNA in vivo is short due to rapid degradation by endogenous nucleases and efficient renal elimination. We will report an efficient formulation of siRNA targeting TNF-α, that was able to restore immunological balance in a mouse arthritis model following intravenous injection. This formulation is also active in an osteosarcoma mouse model and in a model of chronic Hepatotis C, and is progressing toward clinical use.

Biography:

Pr Daniel Scherman is Exceptional Class Director of the CNRS *National Scientific Research Center - France*. **Competence fields**. Drug delivery and targeting, Gene therapy, Non viral Gene delivery, In vivo imaging. **Scientific Production**: h index: 63 (1979-2017) - 16 900 quotations without self-citations - 531 articles in reviewed journals - 50 books chapters - 40 independent patent applications. **Main present functions**: Director of the *Chemical and Genetic Pharmacology and Bioimaging Unit* Partners : INSERM, CNRS, Pharmacy University, Ecole Nationale Supérieure de Chimie de Paris. President of the Committee of "Non Viral Gene Therapy" of the European Society of Cell and Gene Therapy (ESGCT). Member of the Non Viral Gene Therapy Committee of the American Society of Gene Therapy (ASGT). Editor of "Handbook of Gene Therapy and Genetic Pharmacology", Imperial College Press, 2014.

BIOCOMPATIBLE NANOMATERIALS FOR CONSTRUCTION OF VACCINES

Jaroslav Turánek

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Advent of reverse vaccinology together with availability of recombinant technologies for economic massive production of recombinant antigens and mRNA constructs, new biocompatible nanomaterials and molecular adjuvants are prerequisites for successful development and commercialization of new vaccines, e.g. influenza vaccines. Mucosal vaccination represents non-invasive route for immunization having advantage in safety, efficacy and comfort for vaccinees, in general.

Recombinant antigens based on complex nanoparticles like VLP and virus pseudotypes, recombinant protein antigens derived from influenza virus and chimeric multiepitopic or fused complex protein antigens represent valuable tools for development of modern vaccines. Beside intranasal vaccination, sublingual application of vaccines is of growing interest with respect to safety and induction of both systemic and generalized mucosal immune response. Development and application of new mucoadhesive formulations drives forward the development of mucosal vaccines. Great potential is hidden in the use of biocompatible nanomaterials. Polymeric and lipid based nanoparticles are available as carriers for construction of vaccination nanoparticles containing mRNA or recombinant protein antigens. New materials also facilitate a development and use of new technologies for vaccine production.

Multi-layered nanofibrous mucoadhesive films for buccal and sublingual administration of drug-delivery and vaccination nanoparticles - important step towards effective mucosal vaccines. By: Masek, Josef et al; JOURNAL OF CONTROLLED RELEASE Volume: 249 Pages: 183-195 Published: MAR 10 2017

Biography:

Ass. Prof. RNDr. Jaroslav Turánek, PhD., has his expertise in nanotechnology focused on drug delivery systems based on liposomes for construction of self-assembled vaccination nanoparticles and molecular-based adjuvants. He is pioneering the technologies of mucoadhesive formulations for non-invasive mucosal vaccination. Jaroslav Turánek has completed his PhD at the age of 27 years from Masaryk University Brno. He is the head of Department of Pharmacology and Immunotherapy, Veterinary Research Institute, Brno. He has published more than 77 papers on vaccines, drug targeting, anticancer and antimicrobial drugs in reputed journals. He is teaching immunology, biotechnology and immunochemistry at Masaryk University Brno and Technical University Brno. He is the president of Czech Society for Gene and Cell Therapy and Principal Investigator of vast multidisciplinary project OPVVV FIT “Pharmacology, Immunotherapy, nanoToxicology” focused on application of complex nanotechnology and biotechnology approach for development of new modern vaccines and targeted drug delivery systems

POLYSACCHARIDE-BASED SCAFFOLDS FOR TISSUE ENGINEERING AND MOLECULAR IMAGING

Didier Letourneur

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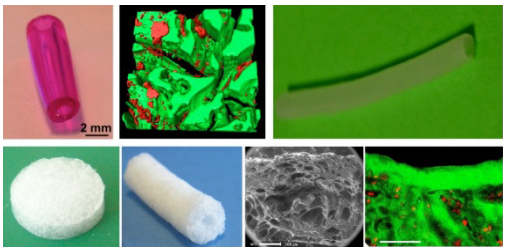
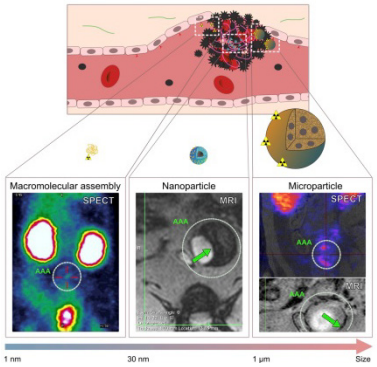
University Paris 13, University Paris Diderot, Paris, France



One main challenge of tissue engineering is to create an optimal environment for growing therapeutic cells to regenerate damaged tissues. This environment can be reconstituted with 3D structures, in which cells can be organized into a tissue-like matrix. We have prepared polysaccharide-based porous 3D scaffolds having controlled pores and porosity for several cell types. These porous hydrogels made of natural biodegradable and biocompatible polysaccharides have architectural characteristics adapted to the cell culture in 3D. We have developed them to different shapes and sizes. Further studies have demonstrated the performance of these matrices for tissue repair in vitro as well as in small and large animals. Examples for heart, vessel, skin and bone will be presented as well as their industrial developments.
Moreover, polysaccharide-based nano and microsystems were also designed and used for the imaging of cardiovascular pathologies as targeted contrast agents for molecular imaging. Examples will be provided using several types of imaging modalities for thrombus detection until Phase I clinical trial.



Biography:

Didier LETOURNEUR, Engineer, PhD in Chemistry, is Research Director at CNRS. He is Director of Inserm U1148 in Paris (http://www.u1148.fr) with about 230 persons. He is involved in several national grants, as European coordinator of NMP "NanoAthero" large scale project, and in several EU projects (“FAD”, “Prestige”, “Nanoantenna”).
He is the author of 180 international publications (h index 35), inventor of 16 patents, and won several prizes : "Coup d’Elan" Bettencourt Foundation 2001, CNRS-Diderot Innovation Award 2009, Cardiovascular Innovation 2011 from FRM, OSEO Emergence 2012, BPI Creation-Development 2013. In 2016, he obtained the G Winter Award the highest distinction from the European Society for Biomaterials, and in 2017 the Asian Polymer Association Jubilee Award. In 2016, he found the company SILTISS for the development of innovative implants.
He has more than 100 invited lectures and was co-organizer of numerous national and international conferences. He is currently President of scientific committee CSS6 Inserm. He is President of BIOMAT, French Society for Biomaterials since 2009.

HYDROPHILIC POLYMERS AS PRECURSORS OF EFFICIENT NANOTHERAPEUTICS

Tomáš Etrych

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Beside the development of novel low-molecular-weight anti-cancer agents, new formulations of "classic" cytostatic drugs, so called drug delivery systems (DDS), including their encapsulation into liposomes and nanoparticles or covalent binding to water-soluble polymers and micelles, appear to be a very promising strategy. Moreover, combination of their therapeutic potential with simultaneous non-invasive diagnostics can yield to highly efficient theranostics, which enable to concurrently observe the disease progression and system pharmacokinetics. Obtained results showed a high potential and capability of nano-sized copolymer-drug conjugates for specific delivery of drugs and their combinations to aggressive solid tumors and thus for their efficient treatment. In the presentation main emphasis will be given to description of potential of water-soluble polymers in the field of nanomedicine. Polymer-based systems, micellar and star polymer-drug conjugates, will be presented and their potential for enhanced passive tumor accumulation and release of drug in the acidic milieu of a tumor will be shown. Moreover, in vivo noninvasive multispectral optical imaging and positron emission tomography of fluorescently or radio labeled polymer carriers will be discussed.

Biography:

Dr. Tomáš Etrych has completed his PhD in Polymer chemistry from Charles University in Prague, now he is a Research Professor of Polymer chemistry and head of Department of Biomedical Polymers of Institute of Macromolecular Chemistry of the CAS. He has published more than 130 papers in reputed international journals, which were cited more than 3500 times and is an author of 9 patents. His research focus is based on preparation and characterization of water-soluble and micellar drug delivery systems for effective treatment of cancer and inflammatory diseases.

HPMA COPOLYMER CONJUGATES FOR EFFECTIVE TREATMENT OF MULTIDRUG RESISTANT TUMORS

Marek Kovář

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Multidrug resistance (MDR) is a common cause of failure in chemotherapy. MDR is either acquired as a result of previous repeated exposure to cytostatic drugs (P388/MDR cells) or naturally, as some tumors are congenitally resistant to chemotherapy (CT26 cells). One of the most common mechanisms of MDR is upregulation of P-glycoprotein (P-gp) expression. Here, we used HPMA copolymer conjugates, whereby the cytostatic drug doxorubicin (Dox) or the derivative of the P-gp inhibitor reversin 121 (R121) or both were covalently bound through a degradable pH-sensitive hydrazone bond. We proved that R121, when bound to a polymeric carrier, is capable of inhibiting P-gp in P388/MDR cells and sensitizing them in relation to the cytostatic activity of Dox. Conjugate bearing both Dox and R121 was found to be far more potent in P388/MDR cells than conjugate bearing Dox alone or a mixture of conjugates bearing either Dox or R121 when cytostatic activity *in vitro*, cell cycle arrest, accumulation of Dox in cells and induction of apoptosis were determined. Importantly, conjugate bearing R121 is also effective *in vivo* as it inhibits P-gp in P388/MDR tumors after i.p. administration. Only conjugate bearing Dox and R121 significantly inhibited P388/MDR tumor growth and led to the significantly prolonged survival of treated mice. However, the most dramatic antitumor activity of this conjugate was found in the CT26 tumor model where it completely cured six out of eight experimental mice, while conjugate bearing Dox alone cured no mice.

Biography:

1994-1999: Faculty of Natural Sciences, Charles University in Prague (graduated from molecular biology, virology and immunology)
1999-2003: Faculty of Natural Sciences, Charles University in Prague; (PhD study in immunology)
2004-2006: Laboratory of Prof. Jonathan Sprent, The Scripps Research Institute, La Jolla, USA (Research Associate), postdoctoral stay
From 1997: employment at Institute of Microbiology, CAS, v.v.i.
From 2006: principal investigator, Laboratory of Tumor Immunology

* research and development of polymeric conjugates based on HPMA copolymers bearing cytostatic drugs
* complexes of IL-2 and anti-IL-2 mAbs as extremely potent modulators of immune reaction
* induction and regulation of anti-cancer immune reaction with primary interest in $CD8^+$ T cells

CYTOSOLIC PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) IN NEUTROPHILS: A NEW TARGET TO DAMPEN INFLAMMATION

Véronique Witko-Sarsat

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Discovery of innovative treatments for inflammatory diseases requires a better understanding of the molecular mechanisms governing the function and fate of neutrophils, key actors in acute and chronic inflammation. At sites of inflammation, neutrophils release reactive oxygen species (ROS) generated by the NADPH oxidase (NOX2) to kill bacteria. NOX2 is composed of membrane proteins (gp91phox, p22phox) and cytosolic proteins (p47phox, p67phox, p40phox and the small GTPase Rac1/2) that assemble upon activation. Although ROS production is important for innate immunity, excessive release induces oxidative stress leading to cell death and tissue injury.
We have described that cytosolic PCNA controls neutrophil survival through its cytosolic association with procaspases. In the majority of cell types, PCNA is an exclusively nuclear protein that regulates processes related to DNA replication. PCNA exists as a homotrimer that encircles duplex DNA forming a ring-shaped clamp. PCNA has no intrinsic enzymatic activity but instead mediates interactions of its protein partners.
Since PCNA serves as a scaffold and controls the function of associated proteins, we used a proteomic analysis to identify partners within the neutrophil cytosol. Using surface plasmon resonance and crystallography techniques we showed that PCNA associated with p47phox, a key sub-unit of NADPH oxidase and this association regulated ROS production. PCNA inhibition by competing peptides decreased NADPH oxidase activation in vitro and in vivo. We have uncovered a unique role of cytosolic PCNA in neutrophils, governing NADPH oxidase activation and restraining apoptosis, important factors promoting and perpetuating inflammation. Targeting this novel mechanism may uncover new anti-inflammatory molecules in the field of “pro-resoluting agents”.

Biography:

VWS has completed her PhD from Université Pierre et Maris Curie, Paris VI in 1995 and her HDR from the Université Paris Descartes in 2002.
VWS is Director of Research (DR1) at INSERM (Institut National pour la Santé et la Recherche Médicale) and Group leader at the Cochin Institute, Paris France in the department of Immunology, Inflammation and Infection (3I) of the team entitled : "Neutrophils and vasculitis.
https://www.institutcochin.fr/departments/3i
VWS is a councilor at the society for Leukocyte Biology and is a member of the Admnistrative Council of the Societé Française d’Immunologie (SFI) ; Coordinator of the « Neutrophil club » created in July 2014 at the SFI.
http://www.sfi-immunologie.com.fr/

PATIENT-DERIVED LYMPHOMA XENOGRAFTS FOR PRECLINICAL ASSESSMENT OF EXPERIMENTAL TREATMENT APPROACHES

Pavel Klener

Institute of Pathological Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic

At the Institute of Pathological Physiology we established a repository of patient-derived xenografts (PDXs) a malignant lymphomas. Exome sequencing by next generation approach confirmed that PDXs keep majority of somatic mutations contained within the original lymphoma population. Immunohistochemistry analysis of PDXs compared to the original lymphoma biopsies revealed similar immunophenotype of lymphoma cells, but no infiltration with non-malignant immune cells including T-lymphocytes, NK cells or macrophages. In addition, murine, but not human vessels were found in PDX tumors. Some of the derived PDXs were already used for in vivo confirmation of experimental therapies, as well as for evaluation of experimental theranostics.
To conclude PDXs are probably the most reliable models currently available for preclinical research of malignant lymphomas with the exception of certain immunotherapy approaches, which require presence of non-malignant immunocompetent cells.

Biography:

Pavel Klener is a hematology specialist and Head of the Lymphoma Lab at the Institute of Pathological Physiology, First Faculty of Medicine, Charles University.
Pavel Klener and his group focuses on preclinical, translational and clinical research of malignant lymphomas.

TARGETING THE ADENYLATE CYCLASES: DEVELOPMENT OF A TOOL FOR SCREENING OF SELECTIVE MODULATORS

Lukáš Kubala [1,2], *Petra Daďová* [1,2], *Jakub Švenda* [2,3], *Kamil Paruch* [2,3]

[1]Institute of Biophysics AS CR, Brno, Czech Republic
[2]ICRC, St. Anne's University Hospital Brno, Brno, Czech Republic
[3]Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic.

Adenylate cyclases (ACs) produce cyclic AMP (cAMP) which plays an important role in intracellular signal transduction pathways. Ten mammalian AC isoforms identified up to date differ in their tissue distribution and mechanisms of regulation. Since ACs are involved in many physiological and pathological functions (e. g., cardiac and bronchial contractility, pain, aging, skeletal muscle physiology and specific types of cancer), their selective modulation may represent a new therapeutic approach for the treatment of these diseases. Interestingly, most AC isoforms (AC1-8) are activated by forskolin (FSK), a labdane diterpene containing a tetrahydropyran-derived heterocyclic ring produced by plant Plectranthus barbatus. Recently, a 24-step synthesis of (±)-forskolin was developed that opened a potential for the preparation of new forskolin analogues inaccessible by semisynthesis (Hylse, 2017).
Thus, we focus on a development, standardization and comparison of screening methods suitable to monitor specific modulation of the enzymatic activity of different isoforms of human ACs. We prepared various clones of HEK293 cells overexpressing distinct isoforms of ACs. The protocol development included optimization of the isolation of membranes from transfected cells, the concentration of membranes in the reaction, the ATP concentration, incubation time and the presence or absence of a phosphodiesterase inhibitor. The method enables profiling of new, fully synthetic derivatives of FSK, which were recently synthesized in our laboratories. We anticipate that the results will help to define regulatory effects of FSK analogues on specific isoform AC and help to identify potential compounds for therapeutic use in wide range of diseases related to pathogenic cAMP signaling alternations including chronic inflammation, autoimmune diseases and other.

Hylse O, Maier L, Kučera R, Perečko T, Svobodová A, Kubala L, Paruch K, Švenda J. A Concise Synthesis of Forskolin. Angew Chem Int Ed Engl. 2017, 56(41):12586-89.

Biography:

Lukáš Kubala has completed his PhD from Faculty of Science, Masaryk University. He is the head of Department of Free Radical Pathophysiology, Academy of Sciences of the Czech Republic. His research is focused on elucidation of molecular mechanisms underlying acute and chronic inflammatory processes, particularly regulatory role of phagocytes in inflammation and development of vascular inflammation. In collaboration with medicinal chemists from Dr. Kamil Paruch group, Dr. Kubala with his colleagues evaluate biological importance of different isoforms of adenylate cyclase and a potential of new compounds specifically modulating their activities as prospective anti-inflammatory drugs.

POLYMERIC NANOFIBERS FOR DRUG DELIVERY AND TISSUE ENGINEERING APPLICATIONS

Olga Janoušková

(Coauthors: Plch J, Venclíková K., Hrabeta J., Eckschlager T., Kopeckova K., Hampejsova Z., Bosakova Z., Sirch J., Hobzova R.,Abelová L., Popelka Š.)

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Polymeric nanofibres attracted attention for medical application due to their ability to mimic architecture of natural human tissue at the nanometre scale. Their microporous structure and high surface to volume ratio allow efficient cell adhesion, proliferation, migration or differentiation depending on the final therapeutic purposes. The most common method of nanofibres fabrication is electrospinning. Beside their broad application for reparative and regenerative purposes in tissue engineering, nanofibrous carriers prepared from biocompatible polymers can be used as a depot of antitumor drugs. The drug release can be tailor made by appropriate settings of nanofibres structures /structural parameters according therapeutic requirements.
The nanofiber carriers based on polylactide acid (PLA) containing cancerostatic drug paclitaxel were evaluated for the local drug delivery and antiangiogenic effects. Their main benefits are enhanced drug concentration at the site of application, influence of neoangiogenesis, and prolonged duration of action /therapeutic impact in tumor tissue and minimization of side effects together with lower overall drug loading. The PLA nanofibers are studied for the soft tissue application. Here, we present the study on PLA nanofibres membranes designed as a support for retina pigment epithelial cells as possible application for the treatment of degenerative retinal disorders.

Biography:

Olga Janoušková has completed her PhD from molecular biology at the Institute of Hematology and Blood Transfusion, Praque, Czech Republic. She is the head of the Department of Biological Models of the Institute of Macromolecular Chemistry, Czech Academy of Sciences. She has published more than 45 papers in reputed international journals, and is an author of 2 patents. Her research focus is focused on the biological evaluation of medicinal polymers and newly prepared polymeric materials for tissue engineering. The main current research here is concentrated on the interaction of various types of polymers as a carrier of anticancer drug/diagnostic agent with cells, changes in cell behaviour and characterization of cell to cell interaction and communication**.**

IN VIVO PHOTOACOUSTIC IMAGING OF TISSUES USING POLYMERIC CONTRAST AGENTS

Peter Keša[a], *Monika Paurová*[b], *Michal Babič*[b], *Ludek Šefc*[a]

[a]Center for Advanced Preclinical Imaging, First Faculty of Medicine, Charles University, Salmovská 3, 120 00 Prague 2, Czech Republic,
[b]Institute of Macromolecular Chemistry, Czech Academy of Sciences, Heyrovský sq. 2, 162 06 Prague 6, Czech Republic

Photoacoustic imaging (PA) is an elegant noninvasive modality in preclinical imaging using nonionizing techniques with a spatial resolution down to several tens of micrometers and a penetration depth of up to 10 mm. A light beam from a focused nanosecond laser of appropriate wavelength is absorbed by the photoacoustic contrast agent (e.g. some fluorescent dyes, metal nanoparticles, haemoglobin and deoxy-haemoglobin) producing localized heating. As a consequence of the local thermal expansion of the photoacoustic agent, electromagnetic waves are transformed into acoustic waves detectable by an ultrasound transducer. Nowadays, PA is particularly used for the *in vitro* and *in vivo* detection of delivered therapeutic substances. Besides fluorescent dyes, polymer coated metal nanoparticles can be successfully used as nanoprobes, being attractive for their variability to specific molecular binding to molecules, e.g. on the cell surface. On the other hand, polymeric materials based on polypyrrole (PPy) hold great promise for photoacoustic detection because of their strong absorption in VIS and mainly NIR region.

Herein we describe a novel photoacoustic contrast agent based on PPy nanoparticles (35 - 100 nm in diameter) that we are planning to utilize in the preclinical imaging of tissues.

Biography:

Peter Keša has obtained his PhD from Pavol Jozef Safarik University in Kosice, Slovak Republic. He is interested in preclinical imaging by photoacoustics as well as two photon photopolymerization focused on the fabrication of matrices for controlled cell adhesion.

NATIONAL INFRASTRUCTURE TO DISCOVER PROBES FOR BASIC AND APPLIED RESEARCH

Jean-Luc Galzi

School of Biotechnology, University of Strasbourg, France

Since 1995, French chemists have started to gather molecules from nationwide academic laboratories working at the interface with life sciences, in a national collection of bioactive molecules that now contains more than 65.000 distinct compounds and c.a. 15.000 extracts. As it contains no commercial compound and exhibits marked diversity (25 universities, 45 laboratories are compound providers), this collection is unique in Europe. It is shipped from a single distribution site either as a whole, or as subsets either predefined for target categories or defined according to specific user criteria. To help achieving compound selection task, the national collection has added chemo-informatics expertise to its skills. This department offers tools to model molecules and proteins as well as their interactions (virtual screening), as well as modeling of physicochemical parameters such as solubility, barrier crossing, pKa, metabolism, toxicity, etc…. The infrastructure also proposes screening technologies provided by a network of platforms with distinct expertise as in target-based, phenotypic, fragment-based screens with NMR, kinase assays, emerging infectious diseases, protein-protein interactions or orphan protein interaction assays, for instance. Once hit molecules are identified, chemists who provided the molecules and biologists who run the screening are invited to collaborate to further develop the most interesting compounds and the infrastructure further offers advice and services in ADME (absorption, distribution, metabolism, excretion) and toxicology. The presentation will overview the infrastructure economic and scientific models and describe a case study to illustrate the process in which small molecules, called neutraligands, inhibit chemokine actions by an original mechanism according to which the target of the bioactive molecule is the chemokine, not its G protein-coupled receptor.

Biography:

Jean-Luc GALZI has completed his PhD in 1987 from Strasbourg University and has been a postdoctoral fellow in the Institut Pasteur Paris before being hired by the Centre national de la Recherche Scientifique (CNRS) in 1990. He is the director of the Institute for Research on Health Biotechnologies at the school of biotechnology of Strasbourg, and is the director of the national research infrastructure ChemBioFrance devoted the “discovery of probes to understand life and treat patients”. He has published more than 100 papers in reputed pharmacology and more generalist journals and is member of the scientific board from the CNRS and other research institutions.

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NOVEL MUTANT MOUSE MODELS ELUCIDATE PATHOGENESIS OF NETHERTON SYNDROME

Petr Kašpárek

Institute of Molecular Genetics of the ASCR, Laboratory of Transgenic Models of Diseases, Prague, Czech Republic

Netherton syndrome is a severe ichtyosis caused by mutations in the *SPINK5* gene encoding the protease inhibitor LEKTI. It is mainly characterized by a disrupted epidermal barrier, chronic inflammation and structural abnormalities of hair shaft. Patients with Netherton syndrome and mouse models deficient for LEKTI exhibit increased proteolytic activity in the epidermis. To elucidate the role of the individual proteases KLK5 and KLK7 in LEKTI deficient epidermis, we applied technology of programmable nucleases to prepare a set of mouse models combining deficiency for LEKTI, KLK5 and KLK7. We observe an improvement of a number of cutaneous symptoms of Netherton syndrom in both double deficient animal models (KLK5xLEKTI and KLK7xLEKTI) and full rescue of Netherton syndrome-like phenotype in LEKTIxKLK5xKLK7 deficient mice. These data provide in vivo evidence that KLK5 and KLK7 are the main cause of epidermal pathologies in Netherton syndrome patients and help us to understand the role of these proteases in the disease. Furthermore, we demonstrated that novel technologies for generation of mutant animal models, such as TALEN or CRISPR/Cas9 provide reliable and cost-efficient tool to study complex gene networks in vivo.

Biography:

Petr Kasparek has completed his PhD from Institute of Molecular Genetics, Academy of Sciences of the Czech Republic. He is deputy head of Transgenic and Archiving module of CCP (IMG). His research is focused on development of technologies used for mutagenesis in vivo and using mutant mouse models to understand rare diseases.

HIGH-COMPLEX COMBINATORIAL PEPTIDE LIBRARY-BASED REPLICAS OF HIV-1 ENV-NEUTRALIZING ANTIBODIES EPITOPES INDUCE ENV-SPECIFIC SERUM ANTIBODIES IN EXPERIMENTAL MICE.

Raška M. [1], *Kosztyu P.* [1], *Kuchar M.* [2], *Barkocziova L.* [1], *Krupka M.* [1], *Czernekova L.* [1], *Masek J.* [3], *Turanek Knotigova P.* [3], *Turanek J.* [3], *Maly P.* [2]

1 Department of Immunology, Palacky University in Olomouc, Olomouc, Czech Republic
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HIV-1 infection belongs to long term global medical problem. One of the most important obstacle in vaccine development is an enormous antigenic variability and unique biochemical properties of the main antigen proposed for future vaccine - HIV-1 envelope (Env) glycoprotein which is responsible for HIV-1 attachment to and entry into a host cell. New strategies in HIV-1 vaccine development were encouraged after identification of HIV-1 Env broadly neutralizing antibodies (bn-Ab) in sera of elite neutralizer, which may limit disease progression. In parallel to newly introduced strategies for vaccine development based on improved native Env structure-resembling recombinant antigens and germline B cell receptors targeting Env imunogens we used our established concept of a high-complex combinatorial library derived from scaffold of 46 amino acid albumin-binding domain (ABD) and, in combination with ribosome display, we targeted well characterized bn-Ab VRC01 and identified several unique VRC01-binding candidates. These ABD variants we used for immunization of experimental mice and after analysis of serum reactivity with HIV-1 Env we identify ABD candidates inducing Env-recognizing serum antibodies. Thus, ABD-derived recombinant mimotopes could serve as an useful molecular clue for potentially more efficient HIV-1 vaccine development.
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Biography:

Milan Raska has completed his Ph.D. at the age of 35 years from Palacky University, Olomouc, Czech Republic. He spent his postdoctoral fellowship at the University of Alabama at Birmingham, Birmingham, AL, USA at the laboratories of prof. Mestecky and prof. Novak. Dr. Raska is currently a Professor of Immunology at Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic. He has published more than 66 papers in peer-reviewed journals. His research is focused on novel approaches in design of recombinant protein-based and DNA vaccines and on liposome-based delivery systems

ADVANCED METHODS FOR PRECLINICAL IMAGING OF SMALL LABORATORY ANIMALS

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In vivo imaging of small laboratory animals has an increasing importance in translational research. The Center for Advanced Preclinical Imaging (CAPI) at the First Medical Faculty of Charles University is focused on multimodal imaging in preclinical research using all available in vivo imaging methods.

We use CT, MRI, and high resolution ultrasound for anatomical imaging. Molecular imaging is performed by positron emission tomography (PET), single photon emission computed tomography (SPECT), optical image (OI), Cherenkov radiation, photoacoustic imaging (PA) and magnetic particle (MPI) imaging. In collaboration with Advacam, we CAPI, the work on implementing of TimePIX detectors developed at CERN for X-ray spectral imaging and for PET, SPECT, and whole-body X-ray fluorescence.

Energy-sensitive X-ray imaging allows to identify materials or tissues with similar radiation attenuation. Different soft tissues are identified based on their spectral response, resulting in a "color" CT image and allowing differentiation of various soft tissues without the use of contrast media. Detectors can be set to record only the preset power range. This can be used to capture PET and SPECT images reduced for noise and increase the signal to noise (S/N) ratio. The results obtained by spectral imaging are demonstrated on phantoms as well as on whole-body mouse images.

Biography:

Assistant Professor Luděk Šefc, Ph.D., is the head of the Center for Advanced Preclinical Imaging (CAPI) at the First Faculty of Medicine, Charles University in Prague, Czech Republic. He has 20 years of experience in experimental hematology. He is focused mainly on mouse hematopoietic stem cell differentiation and self-renewal, particularly during the recovery from radiation and cytostatic injury. In the past few years, he intensively prepared the establishment of the new small animal whole body imaging facility, the first complex multimodal preclinical imaging center in the Czech Republic. CAPI already celebrated two years of existence and along with in-house research, it serves to scientific community not only in the Czech Republic but through participation in EuroBioImaging also to scientists from abroad.

MULTIPARAMETRIC MAGNETIC RESONANCE IMAGING/SPECTROSCOPY AND CARDIOVASCULAR ALTERATIONS IN DIABETES AND OBESITY

Monique Bernard

Aix-Marseille Université, CNRS, CRMBM (Center for Magnetic Resonance in Biology and Medicine), UMR 7339, Marseille France

Diabetes and obesity are associated with an increased risk of cardiovascular disease which can be related partially to the risk factors associated to diabetes but which remain unexplained. Perturbations in myocardial energetic metabolism might represent early alterations in diabetes preceding contractile alterations due to metabolic remodeling associated to insulin resistance. Endothelial dysfunction and impaired smooth muscle relaxation are also early manifestations in diabetes with consequences at different levels of the coronary tree. An important role of fat around and inside the heart has also been recently emphasized. Cardiac dysfunction is also associated to interstitial fibrosis. Multiparametric magnetic resonance including magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) both in animal models and in patients is an ideal tool to investigate the physiopathology in early diabetes. Function, energetic metabolism, sodium homeostasis, myocardial fat, fibrosis and myocardial blood flow can be investigated by cine-MRI, ^{31}P MRS, ^{23}Na and ^{1}H MRS, parametric imaging and perfusion MRI to identify early markers and determinants of the pathology and for follow up of therapies.

Biography:

Monique Bernard, research director at CNRS, head of Center for Magnetic Resonance in Biology and Medicine (CRMBM), Aix-Marseille Université and CNRS, Marseille, France
Monique Bernard obtained her doctoral degree in 1983 at Aix-Marseille University in France. She was hired by CNRS in Marseille in 1985. In 1989-1990 she was research fellow at Harvard Medical School in Boston in the Group of Prof. J Ingwall. Since 2012 she is director of CRMBM and coordinator of the Marseille Network for in Vivo Imaging, one of the 6 French nodes for in vivo imaging. Her research interests are focused on the study of metabolic, physiological and functional alterations in cardiovascular pathologies related to diffuse or localized ischemia (cardiomyopathy (diabetes, obesity), transplantation…) using magnetic resonance imaging (MRI) and spectroscopy (MRS, ^{31}P, ^{23}Na and ^{1}H). She has published more than 120 original papers and 15 book chapter

MULTI-PARAMETRIC FLUORESCENCE MICROSCOPY FOR PROPER IMAGE DATA INTERPRETATION

Aleš Benda

Imaging methods core facility at BIOCEV, Faculty of Sciences, Charles University, Průmyslová 595, Vestec, 252 50, Czech Republic

Fluorescence microscopy offers unique down to single molecule sensitivity and chemical specificity. Despite this in many real-world applications the specific signal carrying the sought-after information can be hard to distinguish from the autofluorescence or other background of the samples. Modern microscopes allow to capture not only the single photon level signals, but also to determine the spectrum, excited state lifetime and polarization of the signal. Utilizing these extra pieces of information allows to precisely identify specific signals form the sample and to perform artefact free analysis. Apart from purifying the signals, multiparametric detection combined with fluorescence probes and sensors enables to measure local microenvironment and interactions within live cells. During the lecture inspiring examples on some of the applications of multiparametric fluorescence microscopy will be shown and explained.

Biography:

Aleš Benda has completed his PhD from Jaroslav Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic. He is the managing scientist of Imaging Methods Core Facility at BIOCEV, Faculty of Sciences, Charles University. He has published more than 40 papers with more than 1200 citations. His scientific work is focused on development, implementation and application of advanced fluorescence-based microscopy methods in biosciences, particularly functional imaging like FLIM and FCS based methods and super-resolution imaging.

MAUROCALCIN ANALOGUES AS MODULATORS OF SKELETAL OR CARDIAC MUSCLE CONTRACTION

Michel Ronjat

L'unité de recherche de l'institut du thorax, Inserm UMR 1087 / CNRS UMR 6291, Nantes, France

The scorpion venom peptide maurocalcine (MCa) is one of the most potent activator of the intracellular calcium channel type 1 ryanodine receptor (RyR1). Among animal toxins, it is atypical by its ability to cross the plasma membrane in order to reach its pharmacological target by rapid diffusion in the cytoplasm. In contrast to animal toxins that target cell surface receptors, MCa is potentially subjected to intracellular post-translational modifications. We reported that MCa carries a consensus protein kinase A (PKA) phosphorylation site on Thr26. This residue can be phosphorylated by PKA in vitro and following cell penetration in cellulo. Molecular modeling indicates that phosphorylation of Thr26 leads to charge neutralization of Arg24, a residue crucial for MCa agonist activity. Unexpectedly, phosphorylation converts the potent RyR1 potentiation properties of MCa into that of an inhibitor. This is the first reported example of phosphorylation of a small animal venom peptide and likewise of a pharmacological reprogramming of a toxin activity by kinase-mediated post-translational modification. Phosphorylated MCa is the first specific RyR1 inhibitor developed so far and represents a lead compound for further development of phosphatase-resistant analogues.

CELLULAR MODELS FOR THE STUDY OF MULTIDRUG EFFLUX TRANSPORTERS, NUCLEAR RECEPTORS AND MIRNAS

Petr Pávek

Department of Pharmacology and Toxicology, Charles University, Faculty of Pharmacy, Czech Republic.

Cellular models offer key instruments in the drug development, evaluation of side effects of drugs or in examination of therapeutic potential of a novel formulation. Cellular models overexpressing so called multidrug resistance protein (P-glycoprotein, MRP2) or breast cancer resistance protein (BCRP) are nowadays widely accepted models for testing interaction of drugs with these efflux transporters in drug developments and in drug interaction studies. Stable transfected cell lines with a luciferase reporter gene are suitable cellular models for testing interactions of candidate compounds, drugs or environmental contaminants with nuclear receptors or transcription factors in target genes regulation. In addition, the luciferase reporter gene assay can be used in determination of miRNA effects on target genes expression.
In the presentation, I will describe cellular models MDCKII-BCRP and AZ-AHR we recently generated. In addition, I will mention cellular models for the study of miRNA-mediated gene regulation and novel hepatocyte models.

Biography:

Petr Pavek is a professor of pharmacology at the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Charles University. His group focuses on molecular pharmacology, aspects of nuclear receptors-mediated gene regulation, clinical pharmacotherapy and pharmacy, pharmacogenetics and drug and formulation development.

TACKLING INFLAMMATION IN SPINAL CORD INJURY

Pavla Jendelová

Institute of Experimental Medicine CAS, Videnska 1083, 142 20 Prague 4, Czech Republic,

Spinal cord injury (SCI) is a devastating condition which is characterized by an extended secondary injury due to the presence of inflammatory local milieu. NF-κB is considered a prototypical proinflammatory signaling molecule, mainly due to the activation of NF-κB by cytokines such as tumor necrosis factor α (TNFα) and others. We investigated the role of NFκB after spinal cord injury in rats, and how it is modulated by the anti-inflammatory compounds curcumin, Epigallocatechin gallate (EGCG), or by transplantation of spinal neural precursors (SPC). Our results demonstrate that secretary TNFα, perhaps via it's receptor, regulates NF-κB signaling pathways in response to inflammatory cascade generated by secondary injury during SCI. Furthermore, alterations in inflammatory responses produced by either treatment with pharmacological agents, or neural progenitor cell therapy that regulate production of inflammatory cytokines, also appears to regulate NF-κB signaling pathways leading to behavioral improvement from SCI and reduction of glial scarring.

Biography:

Pavla Jendelova has completed her PhD from Institute of Experimental Medicine (IEM), Czech Academy of Sciences. She is the head of Department of Tissue Cultures and Stem Cells, IEM. She has published more than 130 papers in reputed international journals, which were cited more than 3000 times (H index 34) and is an author of 5 patents. Her research focus is based on characterization of adult and embryonic stem cells in vitro; cultivation and differentiation of human pluripotent stem cells into a neuronal phenotype, development of nanoparticles for cell labeling suitable for in vivo cell tracking; regeneration and repair of injured spinal cord and stroke lesion using stem cells, anti-inflammatory compounds and cell-polymer constructs designed to bridge lesions of the central nervous tissue.

DEFINING A MINIMAL NUCLEUS: WHAT CAN THE EGG AND EMBRYO TEACH US?

Helena Fulková

Institute of Molecular Genetics AS CR, Videnska 1083, 142 20 Prague 4, Czech Republic

The emergence of a nucleus was a major event in evolution that ultimately discriminated between prokaryotes and eukaryotes and had far-reaching consequences for cell organization and the regulation of cellular processes. However, we still understand surprisingly little about the construction of a functional nucleus under physiological conditions, and therefore it is currently unclear which nuclear components are truly necessary. We suggest that, to understand the basics of how a nucleus is constructed, we must focus on the very beginning of a new life, i.e. on gametes, fertilization and early embryogenesis. Early embryos are a unique and extremely interesting model in the sense that two clearly functionally and structurally different genomes must be "processed" and remodelled into functional pronuclei that share the same cytoplasmic environment. Therefore, intact eggs can be viewed and used as natural mini-bioreactors for processes such as remodelling and reprogramming of nuclei. With the help of state-of-the-art *in vitro* reproductive technologies we can bypass the limitations of currently employed approaches and using intact eggs gives us the opportunity to follow developmental competence and thus, functional outcome when an artificial nucleus is assembled.

Biography:

Helena Fulkova (Fulka) obtained her PhD from the Charles University in 2008 and is currently employed at the Institute of Molecular Genetics, AS CR. Together with colleagues, she has published 38 scientific papers with nearly 700 citations in international journals including *Science, Development, Trends in Molecular Medicine* or *Stem Cells*. HF has a long-standing experience working with mammalian oocytes and embryos, advanced *in vitro* reproductive technologies including micromanipulations, genetic manipulation, and participated in developing some unorthodox approaches such as interspecific nuclear transfer, embryonic stem cell derivation from maturing oocytes or microsurgical removal of nucleoli from oocytes and embryos.

PFAR4: MINI-PLASMIDS DEVOID OF ANTIBIOTIC RESISTANCE MARKERS - FROM DEVELOPMENT TO CLINICAL TRIALS

Corinne Marie

Paris Descartes University, CNRS UMR8258, INSERM u1022,Chimie-Paristech, France

"Non-viral gene therapy approaches mostly involve the use of plasmids as gene vectors. Our group developed a novel gene vector, called pFAR4, which is devoid of antibiotic resistance markers that are generally required for an efficient plasmid production. Our objective was to develop an efficient eukaryotic expression vector that complies with criteria of pharmaceutical grade in order to favor its translation to the clinic.

After development of the pFAR4 technology, the efficiency of the gene vector was first assessed by monitoring luciferase activities after electrotransfer of LUC-encoding plasmids into various tissues. The pFAR4 vector was generally superior to conventional gene vectors (such as kanamycin-resistant plasmids) in various cultured cells, tissues and organs (muscle, skin, tumours, …). In the liver, hydrodynamic pFAR4 delivery promoted a high and sustained transgene expression, unlike control plasmids that mediated heterochromatin formation and transgene silencing.

To favor prolonged transgene expression in eukaryotic cells that tend to loose introduced plasmid upon cell division, the pFAR4 vector was merged with the components of the Sleeping Beauty (SB) transposon system that mediates transgene integration into the genome of transfected cells. In comparison with other gene vectors, this combination was proven to be superior in HeLa, human retinal and iris pigment epithelial cells, as well as CD4+ and CD8+ T cells.

Either with the pFAR4 alone or in combination with the SB transposon, three clinical trials are expected to be launched in the coming months, in the field of an eye disease, a hearing disorder and immunotherapies.

Biography:

C. MARIE has completed her PhD in Biological Sciences at the University of East Anglia, Norwich, UK. Her current position is Assistant Professor. Her field of Expertise is Molecular Genetics, Molecular Biology and Biochemistry. Her research focus is based on (i) the development of non-viral gene vectors ; (ii) Translation of the biosafe miniplasmids to pre-clinical and clinical trials. She co-invented the pFAR technology.

BIOMEDICAL APPLICATION OF DIAMOND-BASED NANOMATERIAL

Veronika Benson

Institute of Microbiology AS CR, Vídeňská 1083, 142 20 Prague 4, Czech Republic

Nanodiamond material possesses unique characteristics enabling its wide use in biomedical research and future clinical applications. We focus in detail on two strategies i) functionalized nanodiamond particles as transfection and monitoring agents of mammalian RNA and ii) boron doped nanocrystalline diamond layers aiming for the development of advanced electrochemical sensors.

To accomplish the first aim we used fluorescent nanodiamond particles and developed a carrier enabling real-time monitoring, successful transfection, and effective intracellular release of antisense RNA. We proved concept of such a nanocarrier in different systems including topical application on diabetic non-healing wounds *in vivo*. We achieved fast wound closure comparable to non-diabetic animals and we believe the construct represents attractive approach in gene therapy.

The second strategy hypothesized that nanodiamonds enable neurons attachment. We tested the interaction of neuronal cells with nanocrystalline diamond possessing different surface chemistry and topography. Here we found that special topography of nanodiamond supports regeneration of adult neurons that are generally difficult to attach. Such a material can be used as electrode for brain stimulation during neurodegenerative disease.

Biography:

Veronika Benson has completed her PhD in the field of Biomedicine at the Charles University and the Institute of Hematology and Blood Transfusion, both in Prague, Czech Republic. As a postdoc of NCI in Bethesda she practiced experimental oncology and later on, in the Institute of Microbiology she has experienced a bit of cancer immunology. Recently she is the head of Laboratory of Molecular Biology and Immunology in the Institute of Microbiology, Czech Academy of Sciences. Her research group implements molecular biology and nanotechnology approaches in order to study the nanomaterial interaction with biointerface and to develop constructs for treatment of different skin pathologies as well as cancer.

Presented posters:

Aleš Benda, *Faculty of Sciences, Charles University, Vestec, Czech Republic*
What do we mean by advanced imaging?

Aleš Benda, *Faculty of Sciences, Charles University, Vestec, Czech Republic*
Get inspired by FIB-SEM

František Hubatka, *Veterinary Research Institute, Brno, Czech Republic*
Mannan-coated nanoliposomes prepared via orthogonal aminooxy lipids-based click chemistry and microfluidic mixing: characterisation of the structure and in vitro biological activities.

Uliana Kostiv, *Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic*
125I-labeled upconversion nanoparticles for multimodal imaging of tissue in vivo

Libor Kostka, *Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic*
Modular Polymer-Based Antibody Mimetics

Lenka Kotrchová, *Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic*
Water-soluble star polymers based on cyclodextrins

Alena Libánská, **,** *Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic*
Star-like biodegradable nanoterapeutics

Josef Mašek, *Department of Pharmacology and Immunotherapy, Veterinary Research Institute, Brno, Czech Republic*
Nanofibre-based mucoadhesive film for oromucosal delivery of therapeutic nanoparticles

Karla Palma-Alejandro and Mariana Veselá, *Center for Advanced Preclinical Imaging, First Faculty of Medicine, Charles University, Prague , Czech Republic*
3D color Doppler ultrasonography as a tool for a fast and non-invasive monitoring of buried free flaps in Vascularized Lymph Node Transfer

Marina Tavares, *Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic*
Polymer drug carriers for immunooncotherapy