

ENCITE 2nd educational workshop on Cells at Work: imaging in health and disease

A peek into the future of cell imaging

Mons/BE, May 18-19, 2010

After last year's great success of the 1st edition, the European Network for Cell Imaging and Tracking Expertise (ENCITE) and the European Institute for Biomedical Imaging Research (EIBIR), joint organisers, were very proud to present the 2nd ENCITE Educational Workshop. The University of Mons/Belgium, ENCITE project partner, with Prof. Robert Muller and his team, hosted the event, which took place on May 18-19, 2010.

The project partners came another step closer to achieving their goals and presented their scientific highlights to a huge audience of more than 100 participants from 16 different countries. The presentations are available for download at: www.encite.org

Based on initial findings and more recent advances in their research, 28 leading experts throughout Europe and Israel presented remarkable insights into new methods of cell imaging:

Session 1: Imaging of pathophysiological processes

The first session of the workshop, chaired by **Mathias Hoehn** from the Max Planck Institute for Neurological Research in Cologne, focused on the current possibilities to apply imaging techniques for the study and evaluation of pathophysiological processes. In an introductory contribution, Hoehn emphasized the advantages of noninvasive imaging. According to him, the possibility to perform longitudinal, follow-up studies on the same patient or experimental animal provides a new quality of information, not accessible with the more conventional and invasive methods. This comes about because the whole individual time profile of the disease evolution becomes available with the noninvasive imaging modalities. Moreover, an essential ethical issue, raised by Hoehn, is that noninvasive imaging modalities allow to substantially reduce the number of necessary laboratory animals while at the same time generating a better understanding of the diseases under investigation.

In a first scientific presentation, **Wilfried Reichardt** from the University of Freiburg, Germany, gave a methodological introduction to the current magnetic resonance imaging (MRI) techniques. He demonstrated their potential with the example of white matter brain diseases such as eurodegeneration and multiple sclerosis. Here, imaging methods such as diffusion tensor imaging can be applied to analyze lesions of nerve fiberbundles and to monitor efficiency of new treatment strategies for the diseases.

Ignacio Melero from the University of Pamplona, Spain, accompanied the audience on a tour into the still new field of gene therapy using viral vectors. He showed that liver gene transfer with adenoviral vectors can be more effectively accomplished under immunosuppression, and demonstrated the value of noninvasive imaging based on PET for the monitoring of gene therapy in patients.

A new imaging method, allowing the detection of vascular networks in the brain at high resolution was presented by **Philipp Böhm-Sturm** from the Max Planck Institute for Neurological Research in Cologne. He shared his enthusiasm with the audience about the formation of new vessels in the affected brain area following stroke, being detectable in small experimental animals. The speaker pointed out the potential of this new technique for the measurement of vessel density to monitor new vessel formation as an important prerequisite for recovery from stroke.

Finally, the last speaker of the first session, **Daniel Jiráček** from the Institute for Clinical and Experimental Medicine in Prague, Czech Republic, introduced a new approach to better detect small cell clusters after their implantation into the recipient. Application of this so-called “positive contrast with echo-dephased SSFP sequence” method permits to visualize even single transplanted pancreatic islets unambiguously, where clear detectability with the conventional imaging approach often remained questionable. This positive contrast technique exploits a novel imaging measurement sequence making use of very fast scanning times on a clinical scanner.

In summary, the first session, successfully covered a broad range of new imaging modalities, with application to a series of disease situations in both man and animal. Rapid technological advancement and fast improved understanding of diseases have been demonstrated to go hand in hand, already with perspectives pointed out that hopefully will lead to better and more efficient therapeutic situations in the near future.

Session 2: Imaging cell functions

The second session on imaging cell functions was chaired by **Peter Friedl** from the Radboud University Nijmegen Medical Centre, The Netherlands. The detection of functional states of cells in the body to discriminate healthy from diseased tissues and organs is an important research field which is about to change future experimental and clinical imaging. Cell functions currently detected are glucose uptake as indicator of metabolic activity, markers associated with cell activation and differentiation and cell surface receptors that are present on particular cell types only, such as neurons or immune cells. Most recently, novel probes were developed to visualize cell proliferation or cell death (apoptosis). In addition, ex vivo labeling of cells used for transfer into patients during cell-based therapy are labeled with stable probes so their distribution in the body and function state can be followed over time. In this session, different approaches to visualize cells in the test tube and in live animals or patients were discussed, ranging from ex vivo labelling of therapeutic cells to novel reporters that visualize specific cell activation and death.

Erik Aarntzen from Radboud University Nijmegen, Netherlands, reported on new approaches used for monitoring immune responses in melanoma patients during immunotherapy using 18F-FDG and 18F-FLT. The patients received antigen-pulsed dendritic cells that stimulate the anti-melanoma response by activating cytotoxic T cells in vivo. The data demonstrates that patients with improved vaccination-induced T cell response show better survival than patients with poor immunoreactivity, which provides a proof of concept for immunotherapy in melanoma. Sensitive imaging of the activation efficiency of immune effector cells in lymph nodes during immunotherapy by 18F-FDG and 18F-FLT PET scanning was established. In direct comparison 18F-FLT showed superior sensitivity to 18F-FDG for monitoring lymph node responses in cancer patients during immunotherapy. Furthermore, 18F-FLT, but not 18F-FDG directly correlates to in vivo induced immune responses to the control KLH, indicating an antigen specific immune response.

Chantal Brüggemann from Max Planck Institute in Cologne, Germany, showed high resolution calibration data on the sensitivity of MRI imaging in vitro up to cellular level so that only few cells could be detected. Glioma cell aggregates in tissue culture were monitored by fluorescence and MRI revealed that as little as five cells can be detected by MRI. Using human kidney cancer cells transfected with CD4 an anti-CD4-SPIO antibody reached an in vitro sensitivity of 100 cells. A similar approach was used to express CD4 in ES-cells and differentiated neurons, together with luciferase, as tool for preclinical in vivo imaging to allow for combined MRI, fluorescence and luciferase imaging in tumour cells and neuronal cells.

Markus Aswendt from the Max Planck Institute in Cologne, Germany, presented new approaches for cell specific MR imaging with responsive contrast agents. Based on previous work with a lipase-dependent responsive contrast agent, which was already successfully applied in vitro and in vivo, further development was made in a collaboration with Silvio Aime (University of Torino). A contrast agent dependent on β -galactosidase and tyrosinase was developed and characterized using MRI in vitro. This contrast agent will be applied in transgenic cells, driving the expression of the tyrosinase under a cell specific promoter, leading to a selection criteria with MRI due to contrast change after enzyme expression. Likewise, a detection system for glutamic acid decarboxylase (GAD) was used to monitor GABAergic neuronal activity in brain tissue lysates. For further studies a new differentiation protocol for murine embryonic stem cells was developed, leading to a high proportion of GABAergic cells after only 10 days. The labeling of these cells was successfully achieved with electroporation

without affecting the differentiation capacity. The novel contrast agent strategies will be useful for MRI during cell and tissue grafting and regeneration.

Natalie Yivgi-Ohana from Weizmann Institute of Science in Rehovot, Israel, developed novel optical imaging approaches to monitor cell apoptosis using the Bcl-2 family member Bax and a fluorescent caspase reporter. An innovative split-YFP Bax-reporter system (Bax-Bax-YFP) was generated to measure Bax homodimerization based on split-fluorescence complementation. The construct resulted in sensitive apoptosis-dependent mitochondrial label in MCF-7 breast cancer cells during chemotherapy with excellent signal-to-background ratio in vitro as well as in tumor xenografts in vivo. As second system, an mCherry caspase sensor was established and functionally tested. Apoptosis in vivo was verified in chicken embryos during neural-tube closure confirming sensitive and non-toxic apoptosis detection in a complex model. Both apoptosis sensors will be instrumental in measuring different pathways during cell apoptosis with relevance for anti-cancer therapy.

Stefaan Soenen from Katholieke Universiteit Leuven, Belgium, showed the use of iron oxide nanoparticles in live-cell systems and their impact on cell viability. The studies focussed on the tolerability of nanoparticles by live cells using magnetoliposomes. Low and high doses of nanoparticles of different sources were tested in neural and endothelial cells for their impact on cell viability, cell proliferation and cytoskeletal organization. All probes moderately impaired cell viability and compromised both proliferation and cytoskeletal organization in direct relationship with the intracellular mass of iron present. Furthermore, the intracellular degradation of the particles due to the acidic endosomal environment was investigated and its effect on MR contrast generation, reactive oxygen species production, cell viability, transferrin receptor expression and functionality of neural progenitor cells. In direct comparison, MLs and Endorem were overall better tolerated than Resovist and especially VSOP. These data set standards for monitoring novel imaging probes and strongly suggest that a set of complementary cell-function approaches should be tested for novel imaging agents.

Session 3: Imaging and Cell Therapy

The main focus of this session was the visualization of cell based therapeutic approaches by using molecular imaging techniques. It was stressed that the high resolution of MRI based methods is a clear advantage over other methods. Cell visualization by MRI after labeling with iron oxide based particles has already entered the clinic as for example demonstrated for the monitoring of implanted pancreatic islets in the liver of patients. Iron oxide based particles are by far the most sensitive MRI contrast agents but have the disadvantage of causing negative and often unspecific contrast. Contrast generated by other physiological processes like immune response or bleedings can be misinterpreted as contrast caused by iron oxide particle labeled cells. To overcome such limitations, efforts are made to use 19-fluorine (19F) based contrast agents for cell monitoring. Whole body 19F MRI has the advantage of having no background signal so that the generated contrast after cell implantation is highly specific. Encapsulated perfluorocarbons have the potential to overcome current sensitivity limitations. MRS based methods have the potential to monitor biochemical differences between different cell types. Although still preliminary, data were presented on in vitro cell cultures that indicate potential biochemical marker compounds. Another approach to overcome limitations of MRI based methods is the combination with optical, nuclear or CT-based methods. Such multi-modal approaches but also monitoring the progress of disease/ therapy at multiple time points require methods that allow co-registration of those often diverse images. Substantial progress has been demonstrated to visualize and analyze such multi-modal and multi-temporal 3D whole body images.

Questions focused around the two key corner stones of non-invasive monitoring of cell therapy:

- (1) Is the generated contrast high enough to visualize small amounts of cells?
- (2) Is the generated contrast specific enough for the visualization of cells in vivo?

Mangala Srinivas from RUNMC, The Netherlands, gave a talk about novel imaging agents that can be tailored to the application and are suitable for clinical use. The talk showcased recent in vivo data utilizing dual 19F MRI and fluorescence imaging, with labeled human dendritic cells. These dendritic cells are identical to those currently used in clinical vaccinations. The application of 19F MRI for quantitative cell tracking from in vivo image data was also discussed, as this is a novel technological development.

In the third presentation, **Boudewijn Lelieveldt** from the Leiden University Medical Center, the Netherlands, explained several developments on image analysis of follow-up small animal imaging studies. He presented an approach to correct for differences in animal posture between follow-up CT scans, making it easier to track changes in anatomy caused by disease processes or by cell therapy. Also, a novel way to browse through the image information in follow-up scans was presented, enabling a more intuitive visual exploration of large amounts of image data.

Bhavana Solanky from Kings College London, UK, delivered a presentation showing the potential of MRS based methods to characterize neural stem cells. This showed the potential of this technique to only to find a biomarker for neural stem cells but also to follow the biochemical changes longitudinally as these cells evolve.

Monika Dezortova from the Institute for Clinical and Experimental Medicine, Prague, Czech Republic, presented an examination protocol that has been developed under ENCITE project for MRI examination of patients treated by labeled Langerhans islets (LO) transplantation in Prague. Sequences used for LO visualization were compared and their (dis)advantages were discussed.

Session 4:

Nanosized probes, tools for contrast enhancement and for imaging-guided therapy

The session focussed on the use of nanosized probes as tools for contrast enhancement and for imaging-guided therapy, covering either basic aspects of nanoparticles' preparation, in vitro characterizations and applications in diagnoses and therapy of major diseases.

In his introductory lecture **Silvio Aime** discussed recent achievements in the field of liposomes as carriers for imaging reporters and drug molecules as well as the potential associated to the use of endogeneous carriers, such as Apoferritin and LipoProteins. He showed how the sensitivity issues intrinsically associated to MRI can be tackled with the use of nanosized carriers that allow the delivery of a high number of imaging reporter units at the site of interest. Moreover new directions that aim at developing new agents that simultaneously act as diagnostic and therapeutic tools ("theranostics") have been open in recent years. In this context interesting results have been reported concerning a dual MRI/NCT probe. NCT (Neutron Capture Therapy) is effective only when a sufficient amount of Boron containing compound has been accumulated at the diseased cells. Coupling the Boron containing compound to a Gd-MRI reporting agent it is possible to quantify the amount of NCT compound that has reached the target cells and anticipate a successful therapy.

Jean-Luc Bridot from the University of Mons/BE showed a platform based on superparamagnetic nanoparticles that was developed for non specific cell labelling. It consists in an iron oxide core coated with carboxysilane species. These particles have attractive stability properties which permit to produce, at the gram scale highly concentrated solution up to 50% in wt. of iron oxide. Particles exhibit interesting magnetic properties thanks to a thin stabilization shell. They form aggregates in DMEM which favour the cell interaction and aggregates can be broken in small clusters and/or individual particles in PBS. These properties appear attractive for cell labelling because of the high level interaction with cells and facilitate the washing step. Key questions asked by the audience were concerning the influence of surface properties and cell interaction. Negative surface and the large size of aggregates play probably both a role in the interaction with cells.

Florence Gazeau from the University Paris-Diderot/CNRS discussed the issue of the becoming of magnetic nanoparticles in the mononuclear phagocyte system. She demonstrated that, upon stress or activation, magnetically-labeled cells can release nanoparticles via the shedding of microvesicles. These microvesicles are taken up by naïve macrophages and trigger an intercellular transfer of the magnetic label. They also lead to the redistribution of magnetic label during differentiation of magnetically-labeled monocytes into macrophages. Intercellular transfer of magnetic label, mediated by cell-released vesicles, complicates the picture of nanoparticles outcome in the organism and their use as MRI cell tracers.

Daniela Delli Castelli from the University of Torino, presented a new MRI method for the in vivo analysis of the kinetic of the intracellular trafficking of paramagnetically loaded liposomes. This method has been applied to the comparison of different liposomes formulations. The developed methodology aims at improving the drug delivery efficacy of liposomal carriers.

Jan Kriz from the Institute for Clinical and Experimental Medicine in Prague explained the basic principles of pancreatic islet transplantation. It can provide almost physiological control of glucose metabolism to Type-1 diabetic patients, but the commonly used technique based on direct injection of islets into the liver portal vein is limited by early destruction of the 50% transplanted tissue. The critical need of an alternative site for islets is widely accepted in community of transplant researchers. Therefore the Prague group deal with a formation of an artificial cavity in a subcutaneous space and within a major omentum of diabetic animals using a polymeric scaffold. They showed that a sufficient blood supply is critical for islet engraftment. A macroscopic assessment revealed dynamic changes in structure of connective tissue and vascular network surrounding implanted scaffolds, but the most important is the blood supply to the internal surface of created cavities. The dynamic contrast enhanced MRI (i.v. injection of 0,05 ml Vasovist®) confirmed the excellent perfusion of control kidney tissue, the nice perfusion of omentally formed cavity one week after the scaffold implantation followed by a local degeneration of vessels in course of time. The blood supply to subcutaneously formed cavities was inadequate and can be improved with injection of mesenchymal stem cells.

Kristina Djanashvili from Delft University of Technology stressed the fact that imaging is among the most important tools in the diagnosis of human diseases. The use of nanosystems, capable of combination of imaging and drug delivery abilities is the biggest challenge in the unprecedentedly growing field of nanomedicine. Many colloidal nanocarriers, such as liposomes, have already found their application as drug delivery systems (DDS), facilitating the biodistribution and pharmacokinetics of drugs, or to function as their reservoirs. Nevertheless, a passive accumulation of drug-carriers does not guarantee a therapeutic effect. Therefore, the mechanisms for an active drug transport and release, and application of targeting vectors for tumor recognition should be considered. Additionally, optimization of physical properties of the nanoprobles for imaging purposes is the key for successful development of imaging-guided therapy.

Besides the MRI approach a contribution on the potential of US microbubbles for imaging-guided drug delivery was presented. The huge potential of imaging to foster new therapeutic applications including fields, such as cellular therapy, hyperthermia, Neutron Capture Therapy, etc was also discussed during this presentation.

PhD students, radiologists, biologists, physicists, computer scientists and medical scientists with an expertise in MR, optical imaging, nuclear imaging and probe development agreed that the workshop offered them unique possibilities for interesting discussions and getting in touch with experts and peers in a pleasant atmosphere. Given the growing importance and interest in issues related to cell imaging along with the highly rated quality of the speakers, the workshop was received with enthusiasm.