**News**

**Imaging Centre Symposium**
The University of Liverpool’s new Centre for Preclinical Imaging, supported by an MRC capital grant, will soon be opening to provide preclinical imaging support to the research community. A one day meeting was held on 13th May to inform researchers about the capabilities of the various imaging modalities that will be available in the Centre. The meeting included talks by applications specialists from iThera Medical, Perkin Elmer and Bruker plus academic researchers from the Safety Hub, Liverpool and Edinburgh Universities.

**BHF/BSGCT Meeting 2015**
The UKRMP Hubs will be exhibiting at the joint British Heart Foundation and British Society for Gene and Cell Therapy meeting being held 11-12 June in Glasgow. If you are attending the Meeting, come along to stand 14 to meet PostDocs to find out more about the research within Hubs and the programme as a whole.

**SafeSciMet Course – May 2015**
The SafeSciMet course, *Drug Safety of Stem Cells and other Novel Therapeutics* was recently held in Liverpool in collaboration with the Innovative Medicines Initiative and European Federation of Pharmaceutical Industries and Associations.

These courses are tailor-made to meet the needs of Safety Sciences in all phases of medicines research and development, encompassing safety, ethical, regulatory and societal topics. Emphasis is on holistic, integrative, translational and 3Rs aspects.

The course which included talks from regulatory bodies, industry case studies, Cell Therapy Catapult, and UKRMP Hubs will also result in a review paper to highlight the current trends in safety of stem cell imaging techniques.

**Publications**
April 2015 vol. 4 no. 4 edition of *Stem Cells Translational Medicine* includes the workshop review *Understanding and Assessing the Risks of Stem Cell-Based Therapies*. In this review the group attempt to identify the important safety issues of stem cell therapeutics, assessing the current advances in
scientific knowledge and how they may translate to clinical therapeutic strategies in the identification and management of these risks. The group also investigate the tools and techniques currently available to researchers during preclinical and clinical development of stem cell products, their utility and limitations, and how these tools may be strategically used in the development of these therapies. Ensuring safety through cutting-edge science and robust assays, coupled with regular and open discussions between regulators and academic/industrial investigators, is likely to prove the most fruitful route to ensuring the safest possible development of new products.

**Tailoring the surface charge of dextran-based polymer coated SPIONs for modulated stem cell uptake and MRI contrast** has been published February 26th in *Biomaterials Science*, 2015, 3, 608-616.

Tracking stem cells *in vivo* using non-invasive techniques is critical to evaluate the efficacy and safety of stem cell therapies. Superparamagnetic iron oxide nanoparticles (SPIONs) enable cells to be tracked using magnetic resonance imaging (MRI), but to obtain detectable signal cells need to be labelled with a sufficient amount of iron oxide. For the majority of SPIONs, this can only be obtained with the use of transfection agents, which can adversely affect cell health. A library of dextran-based polymer coated SPIONs have been synthesised with varying surface charge via a co-precipitation approach and their ability to be directly internalised by stem cells without the need for transfection agents investigated.

**Research Focus – Tracking Stem Cells in Kidney Tissue**

*Dr Marie Held*

Kidney disease is a serious and growing problem in the developed world. Increasing numbers of people are approaching and living with end-stage kidney disease. However, transplant waiting lists are long and there is an urgent need for new developments to prevent kidney damage in the first place, repair damaged tissue and to engineer new, transplantable kidneys.

This study is focussing on the repair of kidney damage, looking at stem cells as therapies. Before the stem cells can be introduced into a living system, they have to be screened to establish whether they have the potential to integrate into the tissue of interest. In order to test the cells for their integration and repair potential, they have to be efficiently introduced into kidney tissue and tracked over time. The most homogeneous distribution can be achieved by dissociating the intact organ, adding the stem cells to the solution and re-aggregating the mixture. This method is well established and is routinely used on flat surfaces, culturing the aggregate at an air-liquid-interface, in a quasi 2D setting. We have adapted the protocol to culture the aggregate in a 3D setting, which is physiologically more relevant. We are culturing the spheroidal aggregates and monitor the development of structures, like tubules, inside.
The top panel in Figure 1 represents a mouse embryonic kidney with a number of crescent shaped green highlighted structures. A fluorescent protein is expressed together with another protein that is involved in the development of kidney tubules, therefore highlighting developing structures. The typical kidney shape can be seen as a less bright green signal surrounding the crescent shapes.

The bottom panel of Fig 1. shows a spheroid generated by dissociating the kidney, re-aggregating the cells and culturing the construct for six days. Again, we imaged a number of bright green structures. Therefore, we can conclude that similar developmental processes occur in the spheroid as they do in the intact kidney.

Next, we have to introduce foreign cells into the system. At the stage of total organ dissociation we replace a fraction of the cells with fluorescence expressing test cells and re-aggregate the mixture. This enables us to achieve a homogeneous distribution of foreign cells within a kidney tissue (Figure 2). We can image the spheroid over time while maintaining culturing conditions and therefore follow the fate of the test cells.

Figure 1: Top: 3D representation of an intact embryonic kidney expressing fluorescence along crescent-shaped structures within the organ. Bottom: 3D representation of a spheroid generated by dissociating a kidney as in the top panel and re-aggregating a fraction of the cells followed by a six day culture. We again get areas of bright fluorescence within the tissue. The red signal is to highlight the shape of the spheroid.

Figure 2: 3D representation of a spheroid generated by mixing kidney tissue cells (invisible) with 10% of a fluorescent cell line. Bar: 100µm
The tool that enables us to image and culture these large samples is the Lightsheet Fluorescence microscope (Figure 3). Samples are suspended in a water-based gel and can be rotated freely around one axis, enabling imaging from various angles. Only a portion of the sample is illuminated reducing the photobleaching and phototoxic effects to a minimum and enabling a much better depth of imaging compared to other microscopy techniques.

Figure 3: Principle setup of a dual illumination lightsheet fluorescence microscope. The illumination and detection are split up into two distinct optical paths with the illumination axis being orthogonal to the detection axis. The sample is suspended in a hydrogel cylinder and can be freely rotated around the vertical axis.

This work will be presented at the Nephrotools 3rd International conference (Liverpool, UK, 8-10 September) and at the 2nd Lightsheet Fluorescence Microscopy International Conference (Genoa, Italy, 5-8 July).

Meet the Team

This issue we hear from Safety Hub PostDoc, Ioannis Bantounas.

Dr Ioannis Bantounas, University of Manchester

I am a molecular biologist and senior postdoc in the group of Prof. Sue Kimber at the University of Manchester. I obtained my BSc in Cell and Molecular Biology (1st Class Honours) from University of Essex, completing my PhD at University of Bristol, developing adenoviral vectors for the expression of small RNAs (including ribozymes and shRNAs) in the central nervous system. I continued as a post-doc, studying microRNAs in the context of neuronal stress/neurodegeneration and developing lentiviral vectors for their expression. Following a brief stint at the National Hellenic Research Foundation in Athens, Greece, working on the role of miRNAs in colon cancer and EMT, I joined the University of Manchester to investigate novel roles of signalling scaffold proteins in axonal growth and transcriptional control of gene expression (protein-coding and miRNA) during glutamate excitotoxicity in neurons.

My current interests include:

- Utilising CRISPR-Cas9 technology for reporter gene insertion in hESC lines under the control of tissue-specific promoters. Emphasis is given to genetic loci marking steps along the kidney differentiation pathway.
• Developing lentiviral vectors to transfer fluorescent and other probes into hESCs for in vitro and in vivo
• Using modifications of the CRISPR system to control endogenous gene expression to more efficiently direct differentiation of hESC to kidney cell types.
• Studying the role of miRNAs in hESC differentiation and controlling kidney development.

Publications


