Organoids & 3D Tissue Models Workshop

Thursday 24 January 2019, 2.00pm

UCL Eastman Dental Institute, 256 Gray's Inn Road, London, WC1X 8LD

1.30pm – Registration EDI Café
2.00pm – Dr Francesco Saverio Tedesco, Welcome and Opening Address
2.05pm – Professor Vivek Mudera, The Discoveries Centre for Regenerative & Precision Medicine
2.10pm - Session 1: Lecture Room 1, EDI

2.10pm – Talk 1: Dr Cláudia Miranda, University of Lisbon, Towards multi-organoid systems for drug screening applications
2.35pm – Talk 2: Dr Cristina C. Barrias, University of Porto, Guiding 3D cell-assembly for (micro) tissue engineering
3.00pm – Talk 3: Dr Umber Cheema, UCL, Development of 3D Tumouroids to interrogate the tumour stroma boundary
3.25pm – Talk 4: Dr Darren Player, UCL, Talk title TBC
3.35pm – Tea/Coffee break, EDI Café
4.05pm – Session 2: Lecture Room 1, EDI

4.05pm – Talk 1: Professor Peter Zammit, King’s College London, 3D artificial muscle constructs for modelling skeletal muscle in health and disease
4.30pm – Talk 2: Professor Patrizia Ferretti, UCL, 2D versus 3D human neural stem cell cultures for neural injury and disease modelling
4.55pm – Talk 3: Dr Laura Pellegrini, MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Development and characterisation of functional choroid plexus organoids
5.05pm – Talk 4: Daniel Holder, UCL Great Ormond Street Institute of Child Health, Using gene-editing and human pluripotent stem cell-derived retinal organoids to generate a potential source of donor cone photoreceptors for transplantation
5.15pm – Talk 5: Dr Laura Novellasdemunt, The Francis Crick Institute, Oncogenic mechanism of APC-truncating mutation in human colorectal cancer
5.25pm – Session 3: Roundtable Discussion, Lecture Room 1, EDI

5.55pm – Closing remarks

6.00-7.00pm – Reception, EDI Café

Find out more about the Discoveries Centre at http://www.thediscoveriesctr.eu/
The project has received funding from the European Union's Horizon 2020 research and innovation programme, under grant agreement No 739572
**Speaker Abstracts & Biographies**

**Session 1:**

**Dr Cláudia Miranda**  
University of Lisbon  
**Towards multi-organoid systems for drug screening applications**  
There is only a small percentage of novel drug candidates that succeed and reach the end of the drug discovery pipeline, which is mainly due to poor initial screening and assessment of the effects of the drug and its metabolites over various tissues in the human body. For that, emerging technologies involving the production of organoids from human pluripotent stem cells (hPSCs) and the use of organ-on-a-chip devices are showing great promise for developing a more reliable, rapid and cost-effective drug discovery process when compared with the current use of animal models. In particular, the possibility of virtually obtaining any type of cell within the human body, in combination with the ability to create patient-specific tissues using human induced pluripotent stem cells (hiPSCs), broadens the horizons in the fields of drug discovery and personalized medicine. Here, we address the current progress and challenges related to the process of obtaining organoids from different cell lineages emerging from hPSCs, as well as how to create devices that will allow a precise examination of the *in vitro* effects generated by potential drugs in different organ systems.

**Dr Cristina C. Barrias**  
i3S-Instituto de Investigação e Inovação em Saúde/ INEB-Instituto de Engenharia Biomédica  
University of Porto, Porto-Portugal  
**Guiding 3D cell-assembly for (micro) tissue engineering**  
Bioengineered microenvironments can be used to promote 3D cell assembly under controlled conditions. We have established different types of microtissue-systems to recapitulate tissue-specific morphogenesis and differentiation, and to understand the impact of microenvironmental signals in such processes. In this context, we have been developing cell-instructive hydrogels, ranging from complex multifunctional hydrogels, to “minimal matrices” containing only the essential biochemical/biomechanical signals essential for cells to exhibit their unique self-organizing properties. Engineered microtissues provide powerful tools for gaining insight into the mechanisms by which cells perceive their microenvironment to organize into specific structures, and to understand how these processes can be guided by matrix features and/or the presence of other cell types. Ultimately, we aim to translate this knowledge into the design of advanced cell-based regenerative therapies and 3D in vitro models. This presentation will cover some examples of studies we have been conducting using different types of microtissue-systems.

**Dr Umber Cheema**  
University College London  
**Development of 3D Tumouroids to interrogate the tumour stroma boundary**  
The tumour microenvironment is complex and multi-faceted and thus plays an important role in cancer progression. The matrix composition and architecture of the stroma, as well as stromal cells, all play a role in either preventing, supporting or directing cancer invasion. These physical parameters are not exclusive to the underlying genetics, which are known to drive cancer progression, however the activation of specific genes may well be directed by the physical environment.

We have developed a biomimetic 3D tumouroid model, which allows us to interrogate the tumour-stroma axis. We have engineered primitive vascular networks within our model. The invasiveness of the cancer mass implanted into tumouroids directly affects the complexity of vascular networks formed in the stroma. Highly metastatic cancers form much less complex vascular networks.

By incorporating patient-derived cancer associated fibroblasts, we have been able to study how these cells enhance invasion of cancer cells and re-model vascular networks in the stroma.

**Dr Darren Player**  
Talk Title TBC

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Session 2:

Johanna Prüller¹, Heather Steele-Stallard¹-², Luca Pinton¹-², Shilpita Sarcar², Sara M. Maffioletti², Francesco Saverio Tedesco², and Peter S. Zammit¹
¹King’s College London, Randall Centre of Cell and Molecular Biophysics, Guy’s Campus, London, SE1 1UL, UK
²Department of Cell and Developmental Biology, University College London, WC1E 6DE London, UK

**3D artificial muscle constructs for modelling skeletal muscle in health and disease**

Skeletal muscle is an archetypal adult stem cell model, in which maintenance, growth and repair of functionally specialised post-mitotic cells is achieved by recruitment of undifferentiated precursors. The functional unit of skeletal muscle is the myofibre: a giant syncytial cell maintained by hundreds of post-mitotic myonuclei. The routine needs for myonuclear homeostasis, together with the more sporadic demands for hypertrophy and repair, are performed by muscle satellite cells. These resident stem cells are normally mitotically quiescent in mature muscle, and so must first be activated to undergo extensive proliferation to generate myoblasts that eventually differentiate to provide new myonuclei.

Means to investigate muscle satellite cell function ex vivo include culturing satellite cells while maintained on their associate myofibre, classic 2D culture and more sophisticated 3D artificial muscle models (e.g. Prüller et al. 2018). Such systems allow not only satellite cell function to be explored in health, but also represent models whereby disease can be simulated (Steele-Stallard et al. 2018).

We have also used induced pluripotent stem (iPS) cells from patients with skeletal muscle laminopathies (e.g. Emery-Dreifuss and LMNA-related congenital muscular dystrophy), to model disease phenotypes in vitro. Laminopathies involve mutation in the LMNA gene encoding Lamin A. Together with Lamin B1/2, Lamin A forms the nuclear lamina: a mesh-like structure located underneath the nuclear membrane that maintains nuclear shape and regulates gene expression. iPSC lines differentiated into skeletal myogenic cells and myotubes had disease-associated phenotypes, including abnormal nuclear shape and mislocalisation of nuclear lamina proteins. Modelling in 3D artificial muscle constructs resulted in recapitulation of nuclear abnormalities with higher fidelity than standard 2D cultures, identifying nuclear length as a robust outcome measure (Steele-Stallard et al. 2018). Our observations reveal that patient-specific iPS cells can model cellular hallmarks of skeletal muscle laminopathies in 3D artificial models, laying the foundation for future drug screening platforms and gene therapy programmes.


**2D versus 3D human neural stem cell cultures for neural injury and disease modelling**

The adult human central nervous system (CNS) has very limited regenerative capability, and injury at the cellular and molecular level cannot be studied in vivo. Modelling neural damage and disease in human systems is crucial to identifying species-specific responses to injury and potentially neurotoxic compounds leading to development of more effective neuroprotective agents. Hence our aim was to develop 3-dimensional (3D) cultures using human neural stem cells (hNSCs) and test their potential for modelling neural insults, including hypoxic-ischaemic and Ca²⁺-dependent injury. Standard 3D conditions for rodent cells supported survival and growth neuroblastoma lines used as human CNS models, but not hNSCs. While neural cells displayed species- and cell type-specific behavior in 3D hydrogels, changes in culture architecture altered gene expression in all cells tested. Importantly, response to damage differed in 2D and 3D cultures of both normal and diseased neural cells, and this was not due to reduced drug accessibility. Together, these findings highlight the impact of culture cytoarchitecture on hNSC phenotype and damage response, indicating that 3D models may be better predictors of in vivo response to damage and compound toxicity.

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Dr Laura Pellegrini, Madeline Lancaster
MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Francis Crick

Development and characterisation of functional choroid plexus organoids

The choroid plexus is a highly conserved and surprisingly understudied secretory tissue in the brain. This tissue is enlarged in humans proportionally to our brain size and displays a number of important functions in the brain such as forming a protective epithelial barrier and secreting the cerebrospinal fluid (CSF). The CSF is important for the maintenance of physiological levels of nutrients in the brain, for the transport of signalling molecules and growth factors and for its protective role in the regulation of intracranial pressure. This fluid regulates several aspects of development in two fundamental ways: by exerting ventricular pressure that may be necessary for driving brain expansion and by regulating neural stem cell proliferation and differentiation in the brain. To explore the role of the choroid plexus-CSF system in early stages of human brain development, we recently established a protocol to generate choroid plexus organoids using a combination of signalling molecules that are physiologically present during the stages of development of this tissue. More interestingly, not only do these organoids develop the choroid plexus but they also recapitulate fundamental functions of this tissue, namely secretion and formation of a tight epithelial barrier. We detected the presence of choroid plexus specific water channels and transporters localised on the apical brush border of the choroid plexus epithelium by histological and EM analysis. These tissues displayed tight junctions forming the epithelial barrier, and we noticed the formation of large fluid-filled cysts protruding from the organoids, the contents of which, analysed by mass spectrometry, highly resembles human embryonic CSF. In conclusion, we believe this system represents an excellent tool to study the role of the choroid plexus-CSF system in human brain development.

Daniel Holder, Elisa Cuevas, Julie Treguier, Jane C Sowden
UCL Great Ormond Street Institute of Child Health

Using gene-editing and human pluripotent stem cell-derived retinal organoids to generate a potential source of donor cone photoreceptors for transplantation

Retinal dystrophies characterised by the loss of photoreceptor cells, are candidates for cell replacement therapy. Advances in modelling human retinal development using pluripotent stem cell-derived retinal organoids offer the potential to provide an unlimited source of photoreceptors for transplantation. This is particularly challenging for cone photoreceptors, as they are naturally scarce compared to abundant rod photoreceptors, and in vitro organoid cultures generate similar cell type proportions to the human retina. In this project, we sought to bias the molecular fate of precursor cells and artificially generate higher numbers of human cone cells. NRL encodes a conserved transcription factor expressed in post mitotic rods, and when absent in mice, the cells destined to become rods form S-cone-like photoreceptors instead, albeit maintaining some rod characteristics. We used CRISPR/Cas9 gene editing in a human embryonic stem (ESC) line to introduce a STOP codon in exon 2 of NRL, to generate a loss-of-function allele. Sequencing confirmed the generation of a homozygous targeted clone. The targeted ESCs were differentiated to generate optic vesicles and analysed at a series of stages of differentiation compared to the isogenic parental line. We show the effect of loss of NRL on expression of downstream targets by immuno-histochemical analysis of retinal organoids and analysed the altered expression signatures of the wild type and NRL<sup>−/−</sup> retina.

Dr Laura Novellasdemunt
The Francis Crick Institute

Oncogenic mechanism of APC-truncating mutation in human colorectal cancer

The tumor suppressor gene adenomatous polyposis coli (APC) is mutated in the majority of colorectal cancers (CRC) resulting in constitutive Wnt activation. To understand the Wnt-activating mechanism of APC mutation, we use the CRISPR/Cas9 technology to engineer various APC-truncation models. This allowed us to generate individual endogenous APC mutations targeting WT cell lines and intestinal organoids. We find that the β-catenin inhibitory domain (CID) in APC represents the threshold for pathological levels of Wnt activation and tumor transformation. Mechanistically, CID-deleted APC truncation promotes β-catenin deubiquitination through reverse binding of β-TrCP and USP7 to the destruction complex. USP7 depletion in APC-mutated CRC inhibits Wnt activation by restoring β-catenin ubiquitination, drives differentiation and suppresses xenograft tumor growth. Finally, the Wnt-activating role of USP7 is specific to APC mutations, thus can be used as tumor-specific therapeutic target for most CRCs. Our APC CRISPR mutant cells provide unique tools for direct quantitation of endogenous molecular changes in a single APC mutation.
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