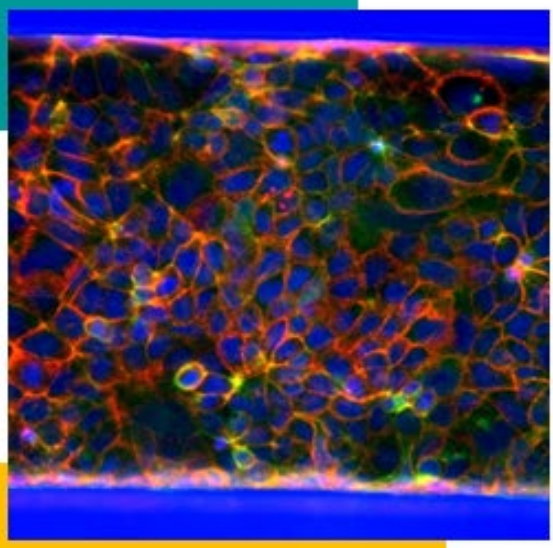
ITN-MIMIC school

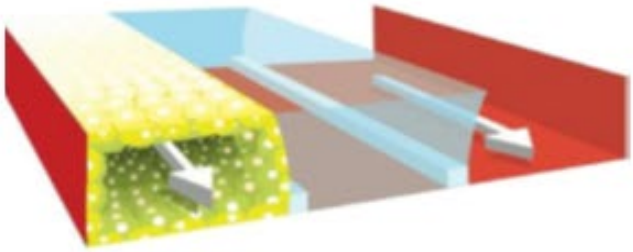
Mimicking organs on chips for high throughput drug screening and basic research

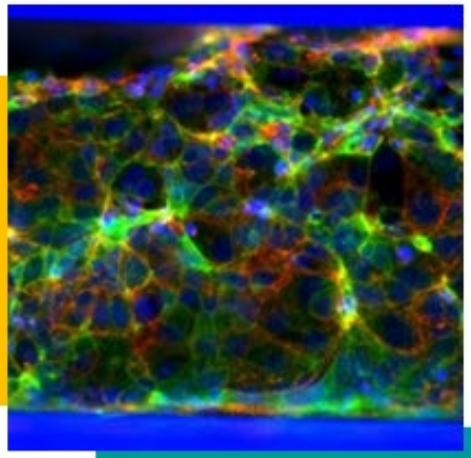


9th April 2019

The Wesley Euston Hotel & Conference Venue

https://www.sheffield.ac.uk/itn-mimic/meetings/school





SCHOOL PROGRAM AND ABSTRACT BOOK

ORGANISERS

The University Of Sheffield.

MIMIC

HORIZON 2020

Trietsch at al. 2017. Membrane-free culture and real-time barrier integrity assessment of perfused intestinal epithelium tubes, Nat Commun, Aug 15;8(1):262

PROGRAM

08:30 – 08:45	MORNING REFRESHMENTS
08:45 – 09:00	ITN-MIMIC introduction – Network coordinator Dr Kai Erdmann
09:00 – 09:50	TALK1: **DR ANIKA NAGELKERKE** (University of Groningen) ***"Technologies for the Advance of Cancer Research"***
09:50 - 10:40	TALK2: **DR MARIA VIAS** (University of Cambridge) ***"Developing Tumour Models of High Grade Serous Ovarian Cancer"***
10:40 - 11:00	COFFEE BREAK
11:00 - 12:30	ITN-MIMIC fellows talks: **KINGA KOSIM** (University of Sheffield) ***''Establishment and Validation of an In Vitro Model for Crohn's Disease''*** **SINDHU NAIK** (University of Sheffield) ***''Establishment and Validation of an In-Vitro Model to Study Lowe Syndrome and Dent II Disease''*** **CLAUDIA BEAURIVAGE** (Galapagos) ***''High Throughput Microfluidic Gut-on-a-Chip Model for Drug Discovery and Target Validation in Inflammatory Bowel Disease''***
12:30 - 14:00	BREAK
14:00 - 14:50	TALK3: **DR CECILE PERRAULT** (Eden Microfluidics/University of Sheffield) ***"Bringing Microfluidics and Organ-on-a-Chip through Commercialisation"***
14:50 - 15:40	TALK4: **DR CHARALAMPOS PITSALIDIS** (University of Cambridge) ***"3D Bioelectronic Devices for the Development of Next Generation Organs-on-Chips"***
15:40 - 16:00	COFFEE BREAK
16:00 - 16:50	TALK5: **PROF JO SPENCER** (King's College London) ***"Human Intestinal Lymphoid Tissue in Time and Space"***
16:50 - 17:00	CLOSING REMARKS AND END

LECTURES

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Technologies for the Advance of Cancer Research

Anika NAGELKERKE, Dr

Faculty of Science and Engineering, University of Groningen

In vitro cell culture systems have generated valuable insights into cellular and molecular biology, both in basic, fundamental science as well as in the context of various disease states, including cancer. However, it has become apparent that two-dimensional models poorly represent the cellular behaviour and responses seen in 3D models and the in vivo situation. For example, several lines of evidence show that drug-responses of cancer cells are highly dependent on the dimensionality of the culture system, with cells cultured in 2D being more sensitive to therapeutics than 3D models. Furthermore, culturing cells of epithelial origin in 2D forces cells to adopt mesenchymal characteristics, whereas in 3D, the epithelial phenotype is retained. The underlying reasons for this behaviour is that 2D cultures do not recapitulate many of the environmental influences cells experience in a tissue-context. This presentation will consider examples of advances in 3D cell culture models for cancer, all aimed at generating more physiologically relevant systems. The importance of extracellular matrix as scaffolds for 3D cell culture will be discussed. Furthermore, microfluidics and on-chip systems will be reviewed in terms of their potential to recapitulate perfused tissues. These systems offer huge potential to assess cellular function in response to therapeutics. By merging analytical techniques with on-chip cell and tissue culture on-line measurements of drug metabolism and cellular responses can be performed. This presentation will show a few examples of organ-chip-based analysis systems which have been implemented in our labs in Groningen.

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Developing Tumour Models of High Grade Serous Ovarian Cancer

Maria VIAS, Dr

Cancer Research UK Cambridge Institute, University of Cambridge

High grade serous ovarian cancer (HGSOC) is the commonest histotype of ovarian cancer and is characterised by extreme genomic complexity and dysregulation of DNA damage repair pathways. Overall survival for women with HGSOC has not improved in the last two decades and five-year survival is less than 30%. The majority of the continuous cell lines used as models of this disease are not HGSOC. There is an urgent need for clinically relevant in vitro models that could be used to identify therapeutic vulnerabilities.

Organoid culture allows the propagation of normal primary human fallopian secretory and ciliated cells for long-term expansion. As HGSOC originates from the secretory cell of the fallopian tube, we established same culture conditions to the propagation of cancer organoids. We considered an organoid line as successfully established when it had been serially passaged >5 times and been cryopreserved/thawed allowing for their long-term storage. We generated 13 HGSOC organoid lines from 10 unique patients and for three patients we have organoids at different time points during patient treatment. HGSOC cancer organoids can be derived at reasonable efficiency and accurately recapitulate genomic and response characteristics of patients.

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Bringing Microfluidics and Organ-on-a-Chip through Commercialisation

Cecile PERRAULT, DR

Eden Microfluidics

Microfluidics is a disruptive technology for manipulation of fluids at microscale. Operating at this scale offers several advantages that can be leveraged in medical devices and as such, microfluidic tools can be envisioned as integrated laboratories. They contain channels with dimensions ranging from single to hundreds of microns that facilitate fluid sensing and reaction, whether it is biofluids (blood, urine, etc.) in a diagnostic device, flue gas for carbon capture, water for micropollutant removal. The gains from such confinement are immense: optimized reagent volumes, boosted reaction rates and higher sensitivity. Microfluidics can also operate from extreme low volume quantities (nanolitre and below) up to several hundred thousand when massively parallelized. Yet, while a number of ingenious microfluidic designs are reported from the academic and industrial research labs, very few of them actually translate into commercial products.

One of the main culprit of this microfluidic dilemma is the lack of a material that permits translation of a prototype into a mass produced system. Currently, prototypes are made of polydimethylsiloxane (PDMS), which is easy to mold, transparent and, to an extent, biocompatible. However, this polymer is incompatible with the mass-production manufacturing methods, who use in large majority thermoplastics (molded by applying heat and pressure). This lack of continuity in material of choice means that microfluidic designs are often developed and optimized for their application in research labs in one material and must again be developed and optimized for mass production in another material. This dual development process is a major restriction for microfluidics to reach its full potential and promises.

In this presentation, Dr Perrault will present an overview of microfluidics and organ-on-chip development, and its current status. She will also discuss a new polymer specifically developed for microfluidics, Flexdym. This elastic, transparent polymer shares many of PDMS advantages, but is compatible with mass production, thus accelerating microfluidics development path.

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3D Bioelectronic Devices for the Development of Next Generation Organs-on-Chips

Charalampos PITSALIDIS, Dr

Department of Chemical Engineering and Biotechnology, University of Cambridge

Animal studies remain the gold standard for predicting the toxicity of new drugs and assessing the efficacy of therapies, however issues due to ethics as well as cost and relevance concerns, have pushed the development of 3D in vitro models that can emulate human physiology and pathophysiology. Advances in 3D cell culture materials and techniques have fostered the development of organ-on-a-chip systems that mimic the structure and the function of native tissues in vitro. While 3D tissues can be generated, sensing technologies that can assess the functionality of these complex models in a dynamic manner is not well compatible with the biological tissue. Taking into consideration the abovementioned challenges, we have developed a 3D bioelectronic organ-on-a-chip based on a conducting polymer scaffold, namely poly(3,4-ethylene dioxythiophene):poly(styrenesulfonate) (PEDOT:PSS), which supports 3D cell cultures and integrates electronic components (i.e., electrodes, transistors) that allow non-invasive electronic readouts of tissue formation. The proposed platform adopts a tubular geometry which allows the facile design of an organ-specific structure while facilitating free flow of nutrients (relevant in a variety of biological tissues (e.g., vascular, gastrointestinal, kidney etc.) and processes (e.g., blood flow)). To validate this technology we have developed an intestine-on-a-chip model based on connective tissue cells (i.e., fibroblasts) and mucus-secreting epithelial cell. The insertion of cells within the scaffold was found to alter the electrical characteristics, while measurements over a prolonged period of time (>3weeks) provides useful information regarding the dynamics of cell growth. This platform represents a first step towards the development of novel multifunctional models (i.e., gut-brain axis) that can be used as predictive cell-based assays for new drugs and therapies.

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Human Intestinal Lymphoid Tissue in Time and Space

Jo SPENCER, Prof

Department of Immunobiology, King's College London

Human memory B cells and marginal zone (MZ) B cells share common features such as the expression of CD27 and somatic mutations in their IGHV and BCL6 genes, but the relationship between them is controversial. In this presentation, Jo Spencer described phenotypic progression within lymphoid tissues as MZ B cells emerge from the mature naïve B cell pool via a precursor CD27−CD45RBMEM55+ population distant from memory cells. By imaging mass cytometry, she illustrates that MZ B cells and memory B cells occupy different microanatomical niches in organised gut lymphoid tissues. Both populations disseminate widely between distant lymphoid tissues and blood, and both diversify their IGHV repertoire in gut germinal centres (GC), but nevertheless remain largely clonally separate. MZ B cells are therefore not developmentally contiguous with or analogous to classical memory B cells despite their shared ability to transit through GC, where somatic mutations are acquired.

MIMIC FELLOWS TALKS

ESR1

Establishment and Validation of an In Vitro Model for Crohn's Disease

Kinga Kosim

Department of Biomedical Science, University of Sheffield

This project aims at providing a tool to investigate Crohn's disease in vitro. Crohn's disease is one of the two major variations of inflammatory bowel disease (IBD) – it is characterized by inflammation and ulceration of the gut mucosa. IBD is a very complex disease with multiple etiological factors associated (genetic, environmental and microbial). The precise mechanism causing the disease is still not uncovered but some genes have been identified to predispose patients for this disease. NOD2 (nucleotide-binding and oligomerization domain 2) has been reported as one of the potential players in Crohn's disease due to its genomic localization and its role in recognition of bacterial cell wall components. Several mutations in CARD15 gene coding for NOD2 protein have been found in Crohn's patients. This protein is part of the innate immune system of intestinal epithelia and its signalling pathway leads to regulation of NFκB activation, which is disrupted in Crohn's disease.

To model Crohn's on a chip I am using an OrganoPlate® developed by Mimetas. The platform based on a microtiter plate harbours up to 96 chips and enables the culturing of perfused 3D tube-like structures in a membrane-free manner. In our model we are using both, the gut epithelial cell lines as well as primary material. As the project focuses on NOD2 involvement in the disease, we induce the inflammation state with MDP (muramyl dipeptide) that is a minimal bioactive peptidoglycan motif common to all bacteria and a NOD2-specific ligand.

We established human gut on a chip model that mimics an intestinal barrier inside the microfluidic channel of the OrganoPlate®. Cell polarization, formation of tight junctions and expression of intestinal markers have been confirmed. Importantly, we are able to show the pro-inflammatory reaction of the epithelium in response to the trigger. The final aim of the project is to demonstrate the suitability of the model for high-throughput drug screening.

There is a great need for more reliable in vitro disease models that are suitable for testing drug toxicity and efficiency at early stages of drug development. Having such models in place would greatly impact on drug development costs and increase safety of newly developed medicines.

ESR2

''Establishment and Validation of an In Vitro Model for Lowe Syndrome and Dent II Disease

Sindhu Naik

Department of Biomedical Science, University of Sheffield

Lowe syndrome is a rare X-linked recessive genetic disorder affecting mainly males. The disease affects mainly three organs: eye, kidney and brain. The disease has low prevalence of about 1 in 500,000 people. OCRL gene, encoding an inositol polyphosphate 5-phosphatase, dephosphorylates phosphoinositide like phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2 & PI(4,5)P3 present on cell membranes and other vesicles into PI4P. Mutations in OCRL, leads to the accumulation of PI(4,5)P2 and PI(4,5)P2 causing cataracts, mental disabilities, and kidney dysfunction in Lowe syndrome patients. Another disease which is caused due to the mutations in OCRL is Dent 2 disease. Dent 2 disease affects only the kidney and has only mild or no effect on other organs.

OCRL is known to be expressed throughout the body, however, it only affects the eye, kidney, and brain. Research has shown that OCRL1 is involved in multiple cellular processes such as endocytosis, membrane trafficking and actin skeleton dynamics which might explain the disease affecting multiple organs. The underlying molecular mechanism due to which the disease is caused is still largely unknown. In this ITN-MIMIC PhD project, I have created an OCRL-1 knock-out stable cell line of human proximal tubule kidney cells (HK-2), using CRISPR-Cas9 gene editing technology. Our goal is to grow them in 2D microfluidic channels with constant fluid-flow mimicking the natural human micro-environment. As, the HK-2 cells are derived from human origin, this gives us an edge over other available animal models for Lowe Syndrome. These cells can also be grown in OrganoPlate® , patented by MIMETAS (The organ-on-a-chip company), where cells can be grown in about 96 (2-lane) channels simultaneously and utilized for high-throughput screening for drug targets at Galapagos N.V (a clinical stage biotechnology company), to ameliorate the disease condition by increasing the surface megalin receptors.

ESR4

High Throughput Microfluidic Gut-on-a-Chip Model for Drug Discovery and Target Validation in Inflammatory Bowel Disease

Claudia Beaurivage

Galapagos, The Netherlands

Inflammatory bowel disease (IBD) is a group of chronic relapsing inflammatory diseases of the gastrointestinal tract. Patients suffering from IBD have presently limited options in terms of treatment due to the lack of physiologically-relevant models to study IBD. With the advent of Organ-on-a-chip technology, a few gut-on-a-chip models have been recently developed and show great promise; however most of them use cancerous cell lines which do not reflect the physiology of IBD patients.

This study reports a novel high throughput microfluidic model of the gut composed exclusively of human primary material. Our first experiments show that primary intestinal epithelial cells (IEC) can be grown in a leak-tight polarized epithelial tubule in the OrganoPlate® platform developed by Mimetas. These cells exhibit intact tight junctions and retain the capacity of differentiating into the different cell types of the intestinal epithelium. An inflammatory state can also be created by exposing IEC to a cytokine trigger; IEC will respond by secreting key inflammatory cytokines such as CCL20, IP-10 and IL-8. Finally, we were able to establish a co-culture model between IEC from healthy donors but also from IBD patients together with primary human immune cells to further investigate IBD mechanisms.

Overall, we show a novel 3D gut-on-chip model entirely composed of human primary material that is suitable for high throughput experiments and screenings. We are presently investigating whether CRISPR-engineered epithelial cells can be grown in this device and therefore be a great tool for target validation. We hope that this model offers an increased relevance compared to existing in vitro models and that it will be suitable to validate potential IBD drug targets and implement it in Galapagos' drug discovery process on a daily basis.

The school **"Mimicking organs on chips for high throughput drug screening and basic research"** was organised by ITN-MIMIC.

ITN-MIMIC - is an interdisciplinary European Industrial Doctorate at the interface of cell biology, engineering and drug development. Organs on chips are a new exciting possibility to more closely mimic human organ functionality in vitro than conventional 2D or 3D cell cultures. Organs on chips allow both, the emulation of healthy organs as well as the emulation of specific disease conditions using corresponding engineered or patient derived human cells. Moreover, organs on chips are ideally suited for high-throughput drug screening. The EID-MIMIC will develop novel organs on chips prototypes, and validate their suitability for end-users for high throughput drug screening or basic research. Consequently, the specific scientific objectives of MIMIC are: Genomic engineering of in vitro disease models suitable for organs on chips incorporation, Development and improvement of organs on chips models and Drug development and End-user suitability of organs on chips models. MIMIC's collaborative innovative research program is an integral part of its training program and is further translated into seven state-of-the-art experimental doctoral training stations representing the combined expertise within the consortium (https://www.sheffield.ac.uk/itn-mimic).

ITN-MIMIC has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 674983.