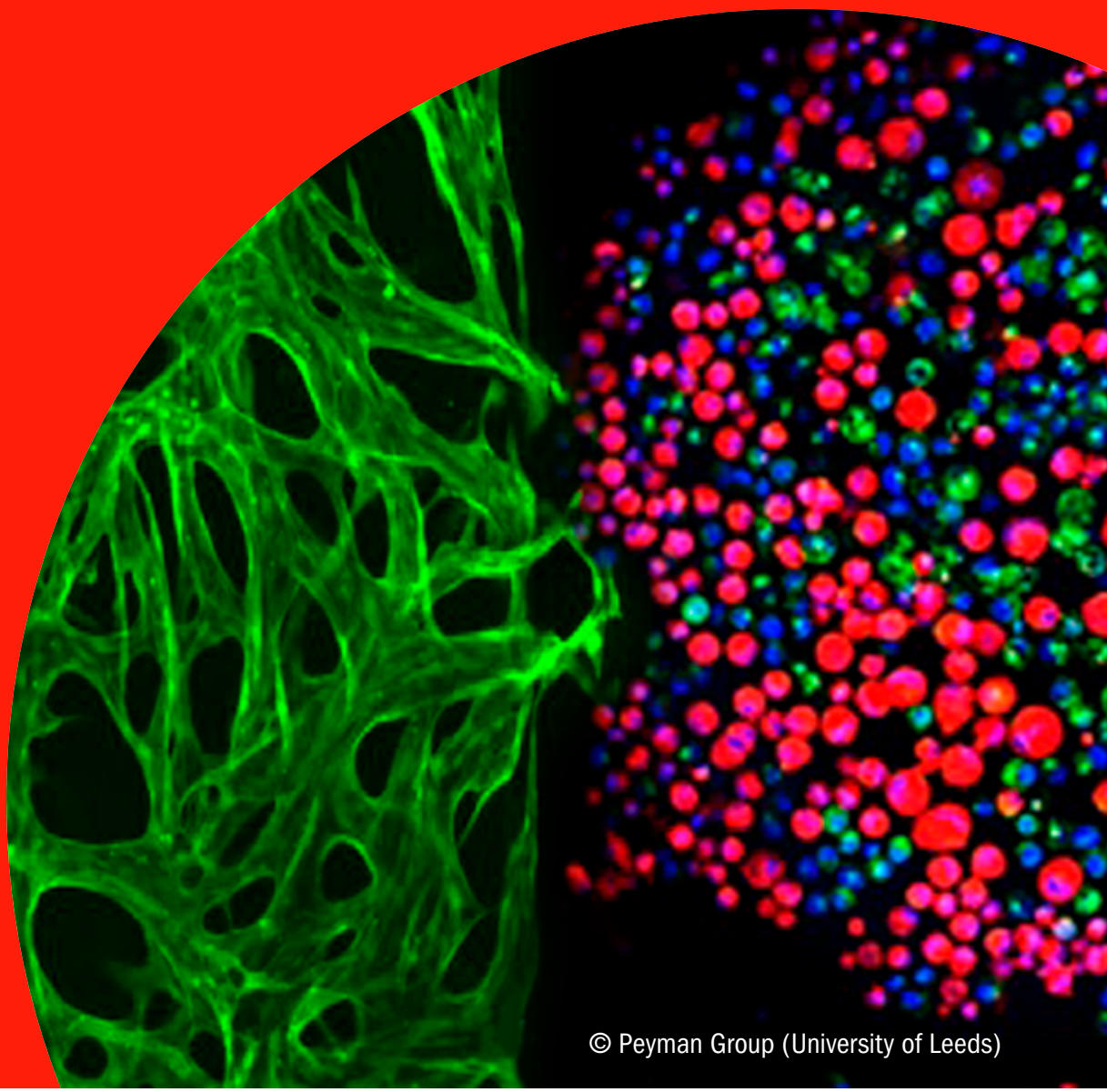
# Organoids/lab on a chip meeting

**21 April 2023**
Institute of Physics, London, UK




iop.org

IOP Institute of Physics

# Invited Talks

## Bioassembling Macro-Scale, Lumnized Airway Tubes via Multi-Organoid Patterning and Fusion

Professor Yan Yan Shery Huang

Invited Talks, April 21, 2023, 11:30 - 13:30

Epithelial, stem-cell derived organoids are ideal building blocks for tissue engineering, however, scalable and shape-controlled bio-assembly of epithelial organoids into larger and anatomical structures is yet to be achieved. Here, a robust organoid engineering approach, Multi-Organoid Patterning and Fusion (MOrPF), is presented to assemble individual airway organoids of different sizes into upscaled, scaffold-free airway tubes with predefined shapes. Multi-Organoid Aggregates (MOAs) undergo accelerated fusion in a matrix-depleted, free-floating environment, possess a continuous lumen, and maintain prescribed shapes without an exogenous scaffold interface. By generating large, shape-controllable organ tubes, MOrPF enables upscaled organoid engineering towards integrated organoid devices and structurally complex organ tubes.

# Downsizing the bladder: a urine-tolerant human urothelial microtissue platform to study infection and to trial novel cancer therapeutics

Professor Jennifer Rohn

Invited Talks, April 21, 2023, 11:30 - 13:30

Diseases of the bladder impose an enormous economic and healthcare burden to society, but are highly understudied. Urinary tract infections (UTI) are among the most common in the world; given their sheer prevalence alongside their tendency to recur, UTI treatment is a critical exacerbating factor in the global antimicrobial resistance crisis. On the other hand, bladder cancer is the 10th most common cancer by incidence in the world. Case rates and deaths have continued to rise year on year, and treatment outcomes have not improved much for four decades. In both cases, therapeutic innovation has probably lagged behind in part due to the reliance on mouse models; although these have yielded great insights, there are nevertheless species differences in urinary tract structure, function, biomarkers and immunity whose consequences to disease relevance are not fully understood.

In recent years, we and others have developed in vitro human cell-based urothelial microtissue models as a complement to mice. Our most recent iteration, 3D-UHU, is planar, three-dimensional and fully stratified to human thickness (up to seven layers, as opposed to the mouse urothelium, which only expresses three); is terminally differentiated with correct biomarkers; has robust barrier function; and is fully urine-tolerant, allowing the exposure of bacteria or drugs in their native environment. 3D-UHU also elaborates a glycosaminoglycan layer on the luminal side, secretes key cytokines in response to environmental stimuli (e.g. bacterial infection), and undergoes physiological cell shedding. We have been using this model to understand bacterial/host interactions at the human cell interface and to trial novel antimicrobial therapies; moreover, we have been downsizing the model further to understand the role of flow and mechanical stretch on the infection process. Finally, we have developed a new platform, 3D-UHU-TU, consisting of human cancer spheroids imbedded in the healthy urothelial environment, for testing the efficacy and safety of novel bladder cancer therapeutics. These approaches illustrate the power of human cell-based microtissue platforms to complement in vivo studies in animal models.

# Microfluidic for embryos and organoids for ART and reproductive biology

Dr Virginia Pensabene

Invited Talks, April 21, 2023, 11:30 - 13:30

Microfluidics and organs on a chip are flexible approaches to confine cells and tissues in submicrometric volumes of medium. These miniaturized systems allow to finely control environmental changes in vitro and thus have the potential to optimized handling, incubation and culture of cells and organoids during essential steps in ART and IVF. In this webinar we will look at essential procedures where microfluidic approaches have been introduced and we will also comment on the complexity of validating and adopting new microfluidic systems in research and clinics.

# Contributed Talks

## Cortical scar-on-a-chip to investigate the effect of electric fields on foreign body response to neural implants

Mr Filip Wronowski[1], Professor George Malliaras[1]
[1]*University Of Cambridge, Cambridge, United Kingdom*

Contributed Talks II, April 21, 2023, 16:00 - 17:00

Implantation of neural implants leads to foreign body response (FBR) within the cortex, which results in development of a glial scar, death of surrounding neurons and decrease in the quality of signal received by the electrodes. A novel approach to decreasing scarring via application of electric field across tissue holds promise in wound healing, however evidence in the context of the central nervous system is lacking.

A new platform for investigating scarring on a chip is necessary for investigation of the behaviour of cerebral cells and tissue under application of electric fields. The platform utilises poly(3,4-ethylenedioxythiophene) doped with poly(styrenesulfonate) (PEDOT:PSS) macro electrodes which provide excellent electrochemical impedance characteristics due to a mixed electronic and ionic conductivity, allowing for long term stimulation across the frequency space.

The initial experimentation includes two-dimensional primary monocultures of astrocytes, fibroblasts and microglia on device assayed at 3-and 7-day in vitro timepoints. To examine cell proliferation, migration and alignment immunocytochemistry staining was performed, and inflammatory status was examined using cell culture supernatant cytokine panel relevant to FBR development in vivo.

Preliminary results suggest decreased cell proliferation and significantly decreased pro-inflammatory cytokine levels for astrocytes stimulated at 1 kHz and increased proinflammatory cytokine levels at 200 Hz and 20 Hz at 3 and 7 DIV. Meanwhile, in meningeal fibroblast stimulation decreased cell proliferation as well as significantly decreased pro-inflammatory cytokine levels at 20 kHz, 1 kHz and 200 Hz stimulation at 3 and 7 DIV. This suggests electrical stimulation as an avenue to alter the development of FBR by altering the rate of cell proliferation and cytokine signalling directly at the wound site.

Current efforts aim to increase the parameter space of stimulation, as well as develop an RNA sequencing approach, which will provide information about transcriptional dynamics and signalling changes induced. Further research will include investigation on organoid or brain slice on chip assays to quantify the changes within the native cytoarchitecture. Lastly, preparations are underway for an in-vivo evaluation of select stimulation parameters on FBR and glial scar development.

# µCOSM - tracking physiological dynamics of individual cells in bulk suspension culture

Dr Somenath Bakshi[1]
[1]*University of Cambridge, Cambridge, United Kingdom*

Contributed Talks I, April 21, 2023, 14:20 - 15:30

Genetically identical populations of microbes are phenotypically heterogeneous. Recent experiments have demonstrated that this phenotypic heterogeneity plays an important role in determining the fitness of microbes in both stressed or unstressed conditions and is therefore a key driver of their evolution over time. To explore and relate the dynamics of microbial populations at single-cell level and at population level, we have developed a microfluidic platform, µCOSM. The µCOSM (microfluidics for Cellular Observation in Suspensions of Microbes) platform enables us to track the physiological dynamics of individual cells as they enter and exit dormant states under complex changing conditions. Using this method and a set of reporters for cellular metabolism, we have found that microbial populations exploit population heterogeneity in their dormant states to hedge their bet for an unforeseen future. For short intervals of starvation, this population heterogeneity is of no consequence, as the benefits of entering and exiting dormant states early is cancelled out by the increased number of progenies of cells that continue to replicate until late commitment to dormancy. However, for an extended period of dormancy, this heterogeneity ensures population survival under stressful conditions (such as antibiotic treatments) and efficient resumption of population-growth under improved conditions.

# Biophysical barriers to drug delivery in cancer

Dr Delanyo Kpeglo[1], Dr Matthew Bourn[1], Dr Sally A. Peyman[1]
*[1]Molecular and Nanoscale Physics Group, School of Physics and Astronomy, University of Leeds, United Kingdom [2]Leeds Institute Medical Research, School of Medicine, University of Leeds, United Kingdom*

Contributed Talks II, April 21, 2023, 16:00 - 17:00

There are many promising new drugs and delivery routes currently in development against cancer. Still there remains a fundamental knowledge gap in the translation of discovery science to the clinic: the biophysical barriers to drug delivery found in the tumour microenvironment. Drugs injected into the body encounter several barriers before ever reaching their target. In the first instance, drugs encounter an endothelial barrier, often distorted by disease. If drugs overcome this first tumour barrier, they are met with a dense, hypoxic, mechanically stiff stroma (Figure 1). High densities of cells and extracellular matrix (ECM) proteins form a rigid mass with high interstitial pressure, blocking drug penetration into the tumour and leading to treatment failure.[1, 2] Yet despite this, most pre-clinical drug trials are performed on cellular models that neglect to incorporate these critical biophysical barriers. Organ-on-chip technology provides a means to mimic tumour biophysical characteristics, allowing accurate test beds to assess new drugs and delivery mechanisms which is not easily achievable in vivo.[2]

Here, we present two barrier on-chip models: 1) a colorectal cancer (CRC) vasculature model displaying the tortuous tumour vasculature, and a 2) pancreatic ductal adenocarcinoma (PDAC) model encompassing high mechanical rigidity and reduced interstitial flow, critical for drug delivery to cells. Our healthy vasculature model was treated with tumour cell media (TCM) taken from CRC HCT116 cells to form the disordered, tortuous tumour vasculature networks (Figure 2). Compared to healthy vasculatures without TCM, these networks over-expressed αvβ3 integrin, a cell-adhesion receptor associated with angiogenesis. This model was used to investigate liposome-vasculature interactions with anti-αvβ3 antibodies towards increasing targeted liposomal drug delivery against distorted tumour vasculatures (Figure 3).[3] PDAC is a cancer hallmarked by rigid fibrotic stroma and poor prognosis. Our PDAC model featured a co-culture of PANC-1 and PSCs, fibroblasts responsible for over-producing collagen, which accounts for the rigid tumour stroma (Figure 4). Off-chip investigations found the PDAC model required a 21-day culture to achieve the collagenous rigid tumour stroma (~1 kPa).[4] This model was translated on-chip to investigate the effect of high culture rigidity on interstitial flow. Culture hydraulic conductivity decreased by day 21 of culture with increased mechanical rigidity (Figure 5). Treatment with the chemotherapeutic gemcitabine (Gem), compared to 2D monolayer cultures (Figure 6), showed how tumour rigidity influence drug delivery to cells.

To overcome these barriers, new drug delivery approaches to tumours are emerging such as microbubbles(MBs)-mediated drug delivery. These micron-sized gas bubbles produce localised shockwaves with ultrasound (US) exposure, creating transient pores in cell membranes and the ECM. These two models were used to demonstrate increased liposomal drug delivery in the tumour vasculatures (by 47%, Figure 3) and Gem effectiveness against the PDAC model (by ~10%, Figure 6), when delivered with US-activated MBs. We demonstrate the importance of modelling disease biophysical characteristics when developing and improving new drug delivery methods.

References

1. Kaemmerer et al. (2021) Drug Discov Today
2. Tian et al. (2022) Journal of Nanobiotechnology
3. Bourn et al. (2023) Lab Chip
4. Kpeglo et al. (2022) Matrix Biology Plus

# Physical Mechanisms of Developmental Symmetry Probed by Magnetic Nanorobots

Dr Fengtong Ji[1], Dr Fengzhu Xiong[1,2]
[1]*Wellcome/Cancer Research UK Gurdon Institute, University of Cambridge, Cambridge, United Kingdom* [2]*Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom*

Contributed Talks I, April 21, 2023, 14:20 - 15:30

A key aspect of development is the creation of diverse tissue and organ shapes (i.e., morphogenesis) which has been extensively studied in vertebrate embryos and has relevance in human diseases such as scoliosis and birth defects [1]. Mechanical characteristics of tissues are of vital importance for the formation of morphological structures in developing embryos. However, for conventional tools or protocols, the in vivo environments of embryos are hard to reach due to the precision needed for smaller measurements and the difficulty in the implementation of sensors. Novel tools that are much smaller and that create small local deformation are required to understand the heterogeneous and dynamic tissue microenvironment of the embryo. The long, symmetric body axis as a key morphogenetic feature forms at an early stage and has long-term functional bearings for the organism (Figure 1). On the other hand, the elongation and thinning of the axis tissues are expected to make the axis more prone to curving (increasing bending stiffness), which could be further exuberated by the soft progenitors that grow the axis in the tail end. In the case of the body axis, it has been shown that the lateral side paraxial mesoderms compress the axial tissues, and the anterior side tissue is dense and stiff [2,3]. However, the mechanical properties of the posterior progenitor domain and body edge cells further posterior are less understood.
Herein, we propose to employ magnetic colloidal micro/nanorobots into early chick embryos to work as interactive sensors and actuators with the cells and tissues. Micro/nanorobots (~1nm to 100μm) are active colloids with functionalised material characteristics [4] and the capability of controllable motion [5]. In particular, magnetic nanorobots are driven by biocompatible magnetic fields, and they can perform distinct motions in environments of different mechanical properties. The progenitors, which undergo epithelial-to-mesenchymal transition (EMT) [6] in the tail bud of chick embryos, may have a drastic change in tissue mechanical properties, as epithelial cells migrate in a tug-of-war manner, whilst mesenchymal cells move more freely. Therefore, we hypothesize that EMT of the tail progenitors guides symmetric axis elongation by specifically reducing the posterior tissue viscosity. Magnetic nanorobots intrinsically form microchains and synchronize with the external magnetic fields. By programming the rotating magnetic fields, these nanorobot chains rotate at a different frequency from the field due to the viscous biofluid and restrictions of tissue barriers. As a result, the synchronization lag with the magnetic fields and oscillation motion pattern (e.g., oscillating amplitude and frequency) of nanorobot chains are used to reveal the in vivo viscosity transition and elasticity of tissue boundaries during the chicken embryonic development. This research is anticipated to elucidate the active mechanism in maintaining axis symmetry during embryonic elongation and the treatment of cancer metastasis.

References:
[1] Cell Dev. Biol., 2016, 55, 119–130. Academic Press.
[2] Nature, 2010, 466(7303), 248–252.
[3] Dev. Cell, 2020, 55(3), 354–366.
[4] Sci. Robot., 2017, 2, eaam6431.
[5] ACS Nano, 2021, 15(3), 5118–5128.
[6] Annu. Rev. Cell Dev. Biol., 2019, 35, 259–283.

# Tissue-Like Sensing Arrays for Long-Term Recording from Cerebral Brain Organoids

Nuzli Karam[1,2], Daniel Lloyd-Davies Sánchez[2,3], Alexander Boys[1,4], Gemma Bale[1], Madeline Lancaster[2], Christopher Proctor[1,5]

*[1]Department of Engineering, University of Cambridge, Cambridge, United Kingdom, [2]MRC Laboratory of Molecular Biology, University of Cambridge, Cambridge, United Kingdom, [3]School of Clinical Medicine, University of Cambridge, Cambridge, United Kingdom, [4]Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge, United Kingdom, [5]Department of Engineering Science, University of Oxford, Oxford, United Kingdom*

Contributed Talks I, April 21, 2023, 14:20 - 15:30

Brain, or cerebral, organoids (COs) show remarkable three-dimensional (3D) mimicry of early brain development.1 3D organoids, however, are limited by lack of vasculature and consequent formation of necrotic cores. COs at the air-liquid interface (ALI-COs) extend the lifetime of COs to give a view into further stages of maturation and development. Recent technological advances in thin-film bioelectronic devices offer high spatial-temporal resolution while minimizing damage to surrounding tissue. The combination of these advances in bioelectronics and the longevity of ALI-COs offers a unique opportunity to study the activity of distinct neural populations long-term, giving new insights into early brain development.

In this work, highly flexible Parylene C-based recording devices with 10 µm thick tissue-like probes and 15 µm PEDOT:PSS coated gold electrodes were fabricated and placed between two CO slices. Preliminary spike activity was recorded, showing similar characteristics to previous reports.2 Devices were tested in ALI-COs for over 3 months. Histological analysis showed minimal glial scarring and no visible impact on the development and health of surrounding tissue. With this work as a proof-of-concept, the long-term stability of such sensing devices can be leveraged with long-lasting ALI-COs, thereby allowing extended recording of electrophysiological activity. Characterization of the later stages of early brain development opens pathways to a deeper understanding of neurodegenerative diseases and human evolution.

1. Lancaster, M., Renner, M., Martin, CA. et al. Cerebral organoids model human brain development and microcephaly. Nature 501, 373–379 (2013).
2. Giandomenico, S.L., Mierau, S.B., Gibbons, G.M. et al. Cerebral organoids at the air–liquid interface generate diverse nerve tracts with functional output. Nat Neurosci 22, 669–679 (2019).Struve, W. S. tetrahalogen substituted quinacridones. US 2,821,530 (1958).

# Flexible Organic Bioelectronics: Enabling Non-Invasive Brain Organoid Electrophysiology at the Air-Liquid Interface

Miss Belquis Haider[1,2], Sagnik Middya[1,2], Daniel Lloyd-Davies Sánchez[3], Ilaria Chiaradia[3], Professor Madeline Lancaster[3], Professor George Malliaras[2], Professor Gabriele Kaminski Schierle[1]
*[1]Department of Chemical Engineering and Biotechnology, University Of Cambridge, Cambridge, United Kingdom, [2]Electrical Engineering Division, Department of Engineering, University of Cambridge, Cambridge , United Kingdom, [3]MRC Laboratory of Molecular Biology, University of Cambridge, Cambridge , United Kingdom*

Contributed Talks I, April 21, 2023, 14:20 - 15:30

Air-liquid interface cerebral organoids (ALICOs) provide a unique opportunity to study brain evolution and development. The air-liquid interface in these models has enabled long-term studies and growth of cerebral organoids as it circumvents issues such as necrosis that limit organoids' growth and functional output. ALICOs have been shown to exhibit robust axonal outgrowth and cortical layering similar to that observed during early development [1]. In this work, we present our development of organic polymer-based flexible microelectrode arrays for non-invasive extracellular electrical activity recording from brain organoids. These devices offer high signal-to-noise ratio, low electrochemical impedance, and match the mechanical properties of cells [2]. We have designed our microelectrode arrays for compatibility with brain organoids at the air-liquid interface, providing a convenient and versatile platform for studying brain activity from ALICOs in situ. We have successfully acquired continuous measurements of spontaneous extracellular activity from brain organoids using our microelectrode arrays and have shown the effects different designs can have on the growth of organoids. Our findings suggest that our flexible microelectrode arrays are a promising tool for long-term non-invasive recording of extracellular activity from brain organoids and will aid in understanding brain development and early disease mechanisms.

References:

[1] Giandomenico, S. L., Mierau, S. B., Gibbons, G. M., Wenger, L. M. D., Masullo, L., Sit, T., Sutcliffe, M., Boulanger, J., Tripodi, M., Derivery, E., Paulsen, O., Lakatos, A., & Lancaster, M. A. (2019). Cerebral organoids at the air-liquid interface generate diverse nerve tracts with functional output. Nature neuroscience, 22(4), 669–679.

[2] Rivnay, J.; Owens, R. M.; Malliaras, G. G. (2014). The Rise of Organic Bioelectronics. Chemistry of Materials, 26, 679– 685.

# Exogeneous Tumour Suppressor Expression Inhibits the Growth and Reduced Bioimpedance of Three-dimensional Lung Cancer Organotypic Models

Xiao Wan[1,2], Jiacheng Xu[3], Cheng Ge[4], Professor Zhanfeng Cui[4]
*[1]Nucleic Acid Therapy Accelerator, Medical Research Council, Didcot, United Kingdom, [2]Target Discovery Institute, University of Oxford, Oxford, United Kingdom, [3]Department of Electronic & Electrical Engineering, University of Bath, United Kingdom, [4]Institute of Biomedical Engineering, University of Oxford, , United Kingdom*

Contributed Talks II, April 21, 2023, 16:00 - 17:00

Early and efficient diagnosis for personalised treatment of pathologies such as cancer remains as a significant topic. Biomarkers have been studied for this purpose to provide early and personalised information on patients. Conventional methods of detecting biomarkers using biopsy samples suffer from limitations such as low sensitivity. Compared with traditional invasive biopsies, non-invasive techniques to detect biomarkers show technical and scientific potential. A sensitive and non-invasive method for early biomarker detection is appealing for quick and efficient personalised diagnosis and potentially treatment. Bioelectronic read-out such as impedance spectrometry may be used. In this study, the tumour suppressor role of a gene which is emerging as a promising biomarker was confirmed using three-dimensional (3D) organotypic cultured models. A bioimpedance measurement device was made using a minimalised design, and our preliminary results show that the read-out of this device can reflect the epigenetic profile of living non-small cancer lung cells cultured in a perfused mini-bioreactor device. Our results demonstrate the possibility of exploiting the non-invasive impedance spectrometry in a physiologically relevant tissue microenvironment, providing a potential new technique for early diagnosis for personalised treatment of certain cancer types including but not limited to lung cancer. Our perfused organotypic models can also provide inspiration for other users in the organoids/lab-on-a-chip community.

# Organ-on-chip platforms for investigating the relationship between tumour rigidity and hypoxia.

Roberto Benard Orci[1,2], Dr Sally Peyman[1], Dr Delanyo Kpeglo[1], Kevin Critchley[1], Evans Stephen[1]
[1]*University Of Leeds, Leeds, United Kingdom,* [2]*CONACyT, Mexico city, Mexico*

Contributed Talks II, April 21, 2023, 16:00 - 17:00

Pancreatic cancer has the worst prognosis of all cancers and is burdened with early, distant metastasis with an overall 5-year survival rate that remains below 10 %. [1,2] It rarely presents any early symptoms and it lacks specific tumour markers, which hinders early detection. Tumours are then diagnosed at late stages presenting tolerance to most treatments. [3]

Pancreatic cancers are characterised by a dense and abundant collagen stroma; the overproduction of extracellular matrix components creates a stiff, scar like, tissue that pushes on its surroundings due to the increased interstitial pressure. Followed by the collapse in vasculature, the tumour is starved of nutrients and oxygen, changing respiration cycle and creating lactic acid as a by-product. Finally, tumour cells then try to escape this environment by metastasizing, which remains untreatable.[4,5] All these mechanisms serve as barriers for drug delivery; the stiff environment, collapse in vasculature, and increase interstitial fluid pressure drive therapeutics away from the tumour, and even when they manage to reach it, they can be deactivated by the acidic environment.

Cancer research has been accelerated by the continuous integration of more sophisticated set-ups to study tumour formation and progression. Organ-on-chips are microfluidic devices capable of replicating some of the most relevant features of the physiology of a given system. Correspondingly, “Tumour-on-chip" (ToC) systems are microfluidic devices that aim to replicate physical aspects of the tumour structure.

This project uses ToC technologies to investigate the mechanical properties of pancreatic cancer and how they relate to its hypoxic landscape. A novel ToC device (Figure 1) will be used to compare the mechanical rigidity of pancreatic cancer co-cultures grown at oxygen environments of 20%, 3%, and 1% (standard incubation, physiologically and pathologically relevant levels respectively ) [6,7],as well as mapping the hypoxic landscape of these co-cultures using a range of new optical and sensor based approaches (Figure 2 shows the fluorescence intensity difference on an ruthenium based oxygen dye that fluorescence in the absence of oxygen). Off-chip viability measurements of the latter are being taken to gain an initial assessment of the co-cultures changes to the different oxygen environments (Figure 3 shows the ATP luminescence intensity of cultures grown at 3% oxygen for 3 weeks).

With the knowledge gained from these experiments, new therapeutic routes for treating mechanically stiff and hypoxic solid tumours will be investigated using the ToC device developed.

References

[1] Parraisa S. et al (2021) Cellular Physiology and Biochemistry
[2] Kleeff J. et al (2016) NATURE REVIEWS, DISEASE PRIMER
[3] Kamisawa T. et al (2016) Lancet
[4] Malandrino A. et al (2018) Extreme Mechanics Letters
[5] DuFort C. et al (2016) Gastroenterology
[6] McKeownS. R. (2014) British institute of radiobiology
[7] Place T. L. et al (2017) Free radical biology and medicine, Elsevier Inc.

# Replicating Key Human Disease Outcomes Using an In Vitro Air-Blood Barrier Model of RSV Infection

Miss Machaela Palor, Dr Robert Hynds, Dr Thomas Benoist, Dr Claire Smith[1]
[1]*University College London, London, United Kingdom*

Contributed Talks I, April 21, 2023, 14:20 - 15:30

BACKGROUND: Respiratory syncytial virus (RSV) causes bronchiolitis and pneumonia in young infants, and there are currently no licensed vaccines or effective treatments available. RSV infection leads to the recruitment of an inflammatory infiltrate known as neutrophils into the airways of infants, and neutrophil-mediated factors such as neutrophil elastase (NE) and interleukin 8 (IL-8) are thought to correlate with disease severity. Clinical studies have also shown that neutrophils found in the systemic circulation of infants with RSV bronchiolitis contain RSV mRNA.

METHODS: Our lab has developed a novel trans-epithelial endothelial model of the human air-blood barrier to study neutrophil behavior and function during RSV infection. We co-cultured primary pediatric airway epithelial cells (AECs) with human endothelial cells (ECs) before infecting them with RSV expressing green fluorescent protein (GFP). After 24 hours, we added human neutrophils obtained from a healthy donor to the basolateral side. We recovered different sub-populations of neutrophils, including basolateral, adherent, and apical (migrated) neutrophils, for subsequent analyses.

RESULTS: Our data show that RSV infection led to a shift in the number of basolateral neutrophils to apical neutrophils, indicating movement across the EC/AEC barrier. Exposure to RSV-infected AECs led to increased expression of NE on basolateral neutrophils compared to the mock-infected control. This was accompanied by an increase in levels of pro-inflammatory chemokine and cytokines, including interferon γ-induced protein 10 (IP-10), interleukin 6 (IL-6), and IL-8, in the apical supernatant of RSV-infected AECs compared to mock-infected control cells.

CONCLUSIONS: Our findings demonstrate that neutrophils present on the basolateral (blood) side of RSV-infected AECs show a similar phenotype to those collected from patients with RSV bronchiolitis. These results replicate key human disease outcomes and indicate that our in vitro model can identify critical mechanisms that mediate epithelial cell damage and promote inflammation in children with severe RSV disease. Future work will investigate the mechanisms behind this and compare the phenotype of neutrophils isolated from our model to those from the blood of RSV-infected infants, allowing us to characterize a neutrophil sub-population that can serve as a biomarker of severe infection.

# Continuously perfusable, customisable, and matrix-free vasculature on a chip.

Lorenzo Veschini[1], Francois Chesnais[2]
[1]*KCL, , United Kingdom,* [2]*Columbia University, New York, USA*

Contributed Talks II, April 21, 2023, 16:00 - 17:00

Creating vascularised cellular environments in vitro is a current challenge in tissue engineering and a bottleneck towards developing functional stem cell-derived microtissues for regenerative medicine and basic investigations. Here we have developed a new workflow to manufacture vasculature on chip (VoC) systems efficiently, quickly, and inexpensively. We have employed 3D printing for fast-prototyping of bespoke VoC and coupled them with a refined organotypic culture system (OVAA) to grow patent capillaries in vitro using tissue-specific endothelial and stromal cells. Furthermore, we have designed and implemented a pocket-size flow driver to establish physiologic perfusive flow throughout our VoC–OVAA with minimal medium use and waste. Using our platform, we have created vascularised microtissues and perfused them at physiologic flow rates for extended time (>2 weeks) observing flow-dependent vascular remodelling. Overall, we present for the first time a scalable and customisable system to grow vascularised and perfusable microtissues, a key initial step to grow mature and functional tissues in vitro. We envision that this technology will empower fast prototyping and validation of increasingly biomimetic in vitro systems, including interconnected multi-tissue systems.

# Development of hydrogel systems for targeted rectal delivery of intestinal cells to injured mucosa in inflammatory bowel disease

Dr Julia Mantaj[1]
[1]*Anglia Ruskin University, Cambridge, United Kingdom*

Contributed Talks II, April 21, 2023, 16:00 - 17:00

Crohn's Disease (CD) and Ulcerative Colitis (UC) are the two main forms of Inflammatory Bowel Disease (IBD). Research recently demonstrated that IBD affects over 1 in 123 people revealing that almost 500 000 people in the UK suffer from these conditions [1]. CD and UC are chronic and progressive conditions, characterised by destructive inflammation of the intestinal tract. Current IBD management consists of drugs including steroids, aminosalicylates, immunosuppressants, and biologics that aim to induce disease maintenance and remission [2]. However, frequent, and systemic drug administration leads to limited local effects, severe side effects, and low patient compliance. Ultimately, approximately 20% of UC and up to 70% of CD patients require surgical colon removal at some point during their disease [3]. Thus, new approaches to locally restore the inflamed mucosa and initiate colonic wound repair are required.
Human intestinal organoids (HIOs) are three-dimensional (3D) cell aggregates derived from primary tissue or stem cells grown in vitro. These 3D entities demonstrate similar composition architecture to the primary intestinal tissue. Studies in mice have shown that HIOs can engraft into injured colonic mucosa resulting in wound repair [4]. Therefore, delivery of HIOs to damaged intestinal epithelium represents a promising therapeutic approach in IBD treatment.
The project aim is to develop hydrogel systems with embedded HIOs and obtain human-relevant information on their efficacy in the restitution of injured colonic mucosa using an improved and human-relevant organ-on-a-chip technology that emulates in vivo intestine physiology, and hence reliably informs the clinical potential of hydrogel/HIO systems.
Initially, a series of hydrogels will be fabricated based on polymers such as methylcellulose and hyaluronic acid. Solubility, swelling, and microscopy experiments will be performed to characterise the systems. Hydrogels will be designed to possess mucoadhesive properties. Subsequently, HIOs will be embedded within hydrogels. The morphology and viability of HIOs following incorporation into hydrogels will be determined using microscopy and cell viability assays. Following this, inflammation will be induced through the administration of lipopolysaccharides to the basolateral side of the colon chip. Hydrogel/HIO systems will be applied to the apical compartment of the chip via flow for 24 hours. Subsequently, the hydrogel/HIO systems will be removed from the chip, and HIO engraftment into injured mucosa will be determined utilising permeability and cytokine release assays. Successful incorporation of HIOs into the intestinal epithelium will result in decreased permeability of macromolecules such as dextran (4 kDa) and a decrease in pro-inflammatory cytokines (e.g., IL-8) in the basolateral chip compartment.
This study will facilitate the development of organoid-based hydrogels that can serve as a safe, effective, and inexpensive therapy (compared to surgery) and significantly improve the treatment of inflammatory bowel disease and increase patients' quality of life.

References
[1] Crohn's & Colitis UK. News Feb 2022; https://www.crohnsandcolitis.org.uk
[2] Pithadia et al Pharmacol Rep 2011;63(3):629-42
[3] Sica et al World J Gastroenterol 2013;19(16):2445-2448
[4] Cruz-Acuña et al Nat Cell Biol 2017;19(11):1326-1335

# Automated real time tracking of cell cycle transitions in brain tumour assembloids links 4N/G2 glioblastoma cells to infiltration

Dr Christopher Yusuf Akhunbay-Fudge[1], Dr Kevin Critchley[1], Mr Ryan Mathew[1], Dr Heiko Wurdak[1]
[1]*University of Leeds, Leeds, United Kingdom*

Contributed Talks I, April 21, 2023, 14:20 - 15:30

Aim

Glioblastoma (GBM) is the most frequent and aggressive primary brain tumour in adults associated with survival rates below 5% despite treatment. GBM recurrence following surgery is inevitable and tumour repopulation is driven by treatment-resistant, residual cells in the surgical cavity. Studying the mechanisms of tumour repopulating cells is challenging due to their molecular heterogeneity and longitudinal brain infiltration dynamics. Single-cell transcriptomic studies have linked GBM heterogeneity to the cell cycle; however, it remains unclear as to what extent cell cycle states affect the infiltrative capacity of GBM. The "go or grow" paradigm predicts that migration and proliferation are separate spatial and temporal events. Here, we used a brain tumour-cerebral organoid invasion (assembloid) approach to test the migratory potential of glioblastoma cell cycle phases during GBM assembloid formation and infiltration.

Methods

Human induced pluripotent cells were differentiated into cerebral organoids that self-assemble with 3-dimensional spheroids of patient-derived GBM cells expressing a fluorescent cell cycle reporter (FUCCI). Through the process of GBM assembloid formation the selective invasion capacity of cycling versus non-cycling GBM cells was quantified by live-cell confocal imaging. Resultant videos were subsequently analysed with an automated image analysis algorithm capable of tracking cell cycle transitions and cellular migration in confocal microscopy planes, enabling the quantification of GBM migration and proliferation over time.

Results

Real time tracking of assembloid formation revealed a proportion of highly infiltrative GBM cells. Assembloids showed GBM infiltration compartments that were reminiscent of residual GBM cells in patient tumour histological sections. Notably, this infiltrative GBM compartment contained a significant number of cycling tetraploid (4N) G2 phase cells that demonstrated a ~1.4-fold increase in directional infiltrative capacity compared to their non cycling (2N) G1 phase counterparts. To isolate and characterize the 4N migratory GBM cell state, we developed a phenotypic separation assay using microenvironmental cues. Specifically, we found that presence of these cues markedly affects GBM migration routes and times enabling the quantification and isolation of the highly migratory GBM subpopulations for further functional characterization and investigation of the 4N/G2 versus 2N/G1 infiltrative cells.

## Conclusion

The combination of the assembloid model and automated imaging analysis has revealed highly infiltrative GBM cells that contain a significant proportion of actively cycling 4N cells, which we hypothesise could be critical to patient tumour repopulation and recurrence following surgery. The migratory potential of a proliferative cell state raises questions about the “go or grow” hypothesis at the level of GBM cellular subpopulations. Further research is needed to define the underlying mechanisms of 4N GBM cell migration; however, the observed cell behaviour may offer a yet unknown GBM vulnerability that could be exploited therapeutically to ameliorate tumour recurrence. Overall, the assembloid model sheds new light onto the link between cell cycle state transitions and infiltrative GBM behaviour providing an avenue towards identifying molecular targets fuelling GBM recurrence.

# Organoids/lab on a chip meeting

21 April 2023

Institute of Physics, London, UK



IOP Institute of Physics