Advances in Cell and Tissue Culture (ACTC) 2022 November 10-11.
Abstracts

Session 1 - NEW APPROACH METHODOLOGIES FOR HUMAN-RELEVANT DRUG DEVELOPMENT

1. **Development of Microphysiological Systems for Oncology: From Small Molecules to Cell Therapies (Keynote lecture)**
Pelin Candarlioglu, GSK, UK

   Key points of lecture

   - Aspects to consider for choosing the right MPS system to assess safety concerns from small molecule-based oncology therapies and give one real world example.
   - How to choose the right model for evaluating efficacy of cell therapies and how qualify them before using for internal decision making?
   - Where do we see the future of MPS in the oncology drug discovery and development?

2. **Modelling normal tissue toxicity using stem cell-derived organoids**
Jia-Ling Ruan, Juri Na, Gabrielle Price, Jessica Gorrill, Iain DC Tullis, Anne E Kiltie, Kristoffer Petersson

   Department of Oncology, University of Oxford

   Clinical radiotherapy trials are often undertaken with little or no emphasis on prior normal tissue toxicity testing. This exposes patients to potential unnecessary toxicity, which diminishes their quality of life and is potentially life-threatening. On the other hand, preclinical testing using animal models often fails to reflect the human response. Stem cell-derived organoids contain multiple cell types and can mimic the *in vivo* structure and function of organs. They offer a clinically relevant alternative for preclinical testing. Here we described the use of murine intestinal organoids and study their response to chemo/radiation treatment compared to *in vivo* assay. We demonstrate that intestinal organoids could reflect the treatment response observed *in vivo*. Future work will focus on building an *in vitro* clinically relevant normal tissue toxicity testing platform using stem cell-derived organoids for oncological treatment optimisation.

3. **Fabrication and development of blood-brain barrier microfluidic chip to emulate the brain vasculature**
Ami Mehta[1,2,3], Anal Desai [2], Dr. David Rudd[2], Dr. Ghizal Siddiqui[2], C. J. Nowell[7], Dr. Ziqui Tong[3], Prof. Darren J Creek[3], Prof. Prakriti Tayalia[2], Prof. Prasanna S Gandhi[6], Prof. Nicolas H Voelcker[3,4,5]

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   The microphysiological systems are human-relevant models used to investigate the drug transport across the blood-brain barrier (BBB) in neurodegenerative diseases. A series of cerebral endothelial cells closely linked to each other with tight junction molecules form the BBB. The formation and maintenance of the BBB is regulated by factors like fluid shear stress (FSS) and other systemic signaling molecules. The brain vasculature exhibits complex heterogeneity across the vasculature

bed, which is challenging to recapitulate in BBB microfluidic models with fixed dimension and rectangular cross section microchannels. Here, we fabricate a Cayley-tree pattern, using lithography-less, fluid shaping technique in a modified Hele-Shaw cell to mimic the brain vasculature in a microfluidic chip. This geometry offers an inherent distribution of heterogeneous FSS within a singular system, due to smooth variations in branch height and width. The hCMEC/D3 endothelial cells cultured in the Cayley-tree designed chip create a semi-elliptical, 3D monolayer of brain endothelium with branching hierarchy, enabling study of the effect of heterogeneous FSS on the brain endothelium. The design and the dimensions of the fabricated BBB-chip mimics the post-capillary venules of the brain. In response to the heterogeneous FSS, the cell actin cytoskeleton undergoes essential remodeling within the same microfluidic chip. The changing phenotypic proteome induced by the heterogeneous FSS show formation and maintenance of a tight barrier due to increase in junctional proteins and transporter protein that influence drug uptake and adaptive drug efflux. We employ our model to study neuroinflammatory conditions by stimulating the brain endothelium with tumor necrosis factor-α (TNF-α) under heterogeneous FSS. This triggers the immune signaling pathway yet, preserving their physiological processes due to shear stress. Our model has immense potential for studies involving drug transport across the BBB, which could be misrepresented in fixed dimension models.
References: Mehta, A., Desai, A., Rudd, D., Siddiqui, G., Nowell, C. J., Tong, Z., Creek, D. J., Tayalia, P., Gandhi, P. S., Voelcker, N. H., Bio-Mimicking Brain Vasculature to Investigate the Role of Heterogeneous Shear Stress in Regulating Barrier Integrity. Adv. Biology 2022, 2200152. https://doi.org/10.1002/adbi.202200152

4. **Understanding Dynamic Immune Responses within 3D in vitro Models of Human Skin**
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The skin is a complex multicellular tissue that contains keratinocytes, fibroblasts, blood and lymphatic vessels and various types of immune cells. Dynamic communication between tissue resident skin cells and circulating immune cells, such as monocytes, is thought to orchestrate the skin's responses to infection and play a role in tissue repair and regeneration. However, the factors that drive monocyte recruitment to human skin during inflammatory events have not been fully characterised. Furthermore, the specific signals that direct monocyte differentiation and fate once within human skin are not known. Current 3D *in vitro* skin models can recapitulate the structure of the skin by fabricating dermal- and epidermal-like layers but do not effectively model the complex and dynamic interactions between the tissue and the immune system. Therefore, the aim of this research is to gain new mechanistic insights into human inflammatory responses, through the development of a novel immune-responsive *in vitro* model of human skin model. Using 3D bioprinting technology, we fabricated and characterised microfluidic hydrogels for the delivery of monocytes within the dermal compartment of 3D skin equivalents. To assess whether the developed *in vitro* model is capable of monocyte recruitment an inflammasome activation assay was established by exposing NTERT keratinocytes within the epidermal-like layer to a lipopolysaccharide (LPS) and nigericin treatment. THP-1 monocytes were injected into the microchannel and monocyte recruitment into the dermal and epidermal layers was monitored at 3 hrs and 24 hrs after treatment. The results showed that treatment using LPS and nigericin stimulated the recruitment of THP-1 monocytes from the microchannel into the skin model and was mediated by signalling from the keratinocytes. These findings demonstrate that the microfluidic skin model has immune responsive capabilities and could be used to investigate human specific inflammatory responses within the skin. On-going studies aim replicate these findings with the presence of an endothelium and with primary human monocytes. Future work will look at monocyte fate and differentiation within the skin model.

5. **Mass-Density and Weight as Novel Biophysical Parameters to Analyse and Sort 3D Cell Culture**
Andrea Cristaldi, Cell Dynamics Ltd, Italy

6. **Cell Health Tools for Drug Discovery - An overview of bioluminescent cell-based assays to monitor cell health**
Darren Heywood, Promega, UK

Session 2 - RESPIRATORY IN VITRO MODELS

7. ***In vitro* respiratory models – from complexity to therapy (Keynote lecture)**
   Doris Wilflingseder, Medical University Innsbruck, Austria

8. **Addition of physiologically relevant conditions and exposures impacting on the toxicity of engineered nanomaterial hazards in an in vitro co-culture of the alveolar region of the lung.**
   Kirsty Meldrum, Joana A. Moura, Joshua W.P. Bateman, Michael J. Burgum, Stephen J. Evans, Shareen H. Doak, and Martin J.D. Clift
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   Many *in vitro* studies focusing upon lung toxicology use models of the lung that are based on healthy lungs using static models and short exposure times. This should be advanced to include physiological characteristics of specific airway regions, including different cells, fluid-flow and membrane flex to mimic breathing. These changes all aim to have a closer physiological resemblance to the human lung, how it is exposed to inhaled matter and to promote the 3Rs. Within our studies we implemented a well-characterised in vitro model of the human alveolar epithelial barrier (A549 epithelial type-II cell and PMA-differentiated THP-1 (dTHP-1) co-culture grown on transwell-membranes at the air-liquid interface (ALI)) to determine if exposure of the particulates, fluid-flow and the "health" of the model would impact the endpoints measured following particle exposure.
   The Quasi-ALI method was initially implemented to determine if single or repeat exposures elicited similar responses before moving towards aerosolisation (VitroCell Cloud12) within the static model. Using the QuasiVivoTM (Kirkstall Ltd.) system, fluid-flow was then applied to the co-cultures and the models exposed to various particulates.
   To also consider an "inflamed" airway (aiming to mimic an inflammatory airway disease), we stimulated our model with type II cytokines before once again exposing to particulate matter.
   This work has mostly focused on the toxicity of pristine nanoparticles, however, these particles do not reflect what we are exposed to in our daily lives. Future work will focus on exposure of these advanced models to standardised indoor air pollution (samples from NIST) particulates, before moving towards real-life particulate matter collected within homes and offices. We then aim to further progress this exposure system by adding known allergens and determining if co-exposure to these impacts endpoints. The physiological advancement of these models is important to help us further understand the potential health effects to these particles.

9. **A 3D Alveolar In Vitro Test System for the Prediction of Chemical Respiratory Sensitizers**
   Sabina Burla1 B. Aline Chary1 C. Pamina Weber1 D. Melissa Saibene1 E. Tommaso Serchi1 F. Jessica Ponder2 G. Kristie Sullivan2 H. Arno C. Gutleb1

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   The increasing prevalence of chemical respiratory allergic diseases resulting in high morbidity deems necessary a precise classification and labelling of chemicals inducing respiratory sensitization, to ensure the hazard is communicated and allow the safe handling and use. The immunological mechanisms underlying the development of respiratory sensitization are not fully understood, and the available new approach methodologies (NAMs) for identification of skin sensitizers fail to categorize chemicals as respiratory or skin sensitizers. Therefore, physiologically relevant test systems for the respiratory tract are essential to anticipate the sensitizing potential of chemicals.
   The aim of the present study was to evaluate the performance of a 3D alveolar-capillary barrier designed for the prediction of respiratory sensitizing chemicals. The model is built using human cell lines: alveolar type II epithelial (A549), endothelial (EA.hy926) and monocytes (THP-1). The cellular arrangement within the test system favors the development of a tissue-like microenvironment by cell-to-cell direct communication and indirectly through the secreted messenger molecules. Additionally, it facilitates the exposure of respiratory sensitizers in a more realistic approach, at the air-liquid-interface (ALI).
   A panel of test items including known respiratory sensitizing chemicals and non-sensitizers was used for exposures to assess the capacity of the test system. Cell viability was measured in the resazurin assay 24h post-exposure at ALI, and the dose-response curves were modelled for the

apical, basolateral compartments, and the complete test system. The test system was subsequently exposed to a test item dose leading to a cell viability of at least 75% and the expression of CD54, CD86 and TSLPr cell surface markers was measured by flow cytometry.
Results show that the test system has the potential to distinguish respiratory sensitizers from non-sensitizers. In addition, tested pro-haptens were correctly identified as respiratory sensitizers, chemicals reported as false negatives by other NAMs for respiratory sensitization evaluation.
References: Chary, A, Serchi, T., Moschini, E., An in Vitro Coculture System for the Detection of Sensitization Following Aerosol Exposure. ALTEX - Alternatives to Animal Experimentation, 36 (3):403-18 (2019).

10. Replicating the alveolar interface in vitro using spherical, transparent, and stretchable membranes

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Human lungs can be modelled using in vitro technologies that enable cell exposure to aerosolized agents. However, they still present some limitations which may alter the study of particle deposition in the lungs and the translation of results to humans [1].
The aim of this project is to overcome these limitations with the design of a breathing acinus. The model will consist of different spherical alveolar structures and an alveolar duct, constituting an alveolar sac. Thanks to the modularity of the system, different sacs can be connected in parallel forming an acinus.
We started from the fabrication of sacs from agarose spherical membranes [2] which replicate the alveolar shape; they are transparent, stretchable, biocompatible, and permeable. The membranes were characterised in terms of tensile mechanical properties during single and cyclic tests (apparent elastic modulus Eapp = 2.6 ± 0.5 MPa at 0.2 Hz); mechanical properties at the nanoscale (compressive Eapp = 201 ± 45 kPa); permeability (comparable to standard polycarbonate membranes with 0.45 µm pores) and biocompatibility (A549 cell adhesion tests). Finally, they were interfaced with an alveolar duct and a blood side obtained via stereolithographic printing (Formlabs Form 2). All the moulds and prototypes were designed using Autodesk Fusion 360.
Using computational models, we verified the physiological oxygenation and shear stress level. The models show that oxygen concentration does not fall below the critical hypoxic threshold (2e^-3 moles/m^3 [3]) and the shear stress does not exceed the maximum physiological value (< 2 Pa [4]). Breathing movements can be also obtained thanks to standard pressurisation methods, in which the airflow is controlled by a pressure regulator.
In conclusion, the prototype developed in this work may contribute to the definition of a physiologically relevant lung model and thus to the reduction of animal testing in line with the 3Rs principles.
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11. A dynamic barrier tissue model system for real-time imaging

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In the human body, the tissues and organs communicate with each other and with the external environment via selective physiological barriers. These barriers are composed of mainly, epithelial cells and connective tissue which serve to compartmentalise the interacting cells, tissues, and organs, and at the same time provide a protective and a nutritive function. Dysfunction of the barrier

leads to pathological consequences. For example, failure of the blood brain barrier is related to many neurodegenerative diseases and weakening of the skin barrier leads to various skin conditions. It is important to replicate these barriers when developing *in vitro* models involving such tissues and organs. Most of the available *in vitro* models utilise removable inserts fitted in cell culture chambers, which can facilitate generation of air-liquid or liquid-liquid interface between the apical and basolateral compartments. Within these compartments, cells or tissues representative of the organ of interest are cultured. However, the current systems do not adequately allow fluid flow on both sides of the barrier, in a physiological manner. The Quasi Vivo® milli-fluidic flow system is an interconnected cell culture chamber system with controllable flow on a mesoscale and is already utilised by many laboratories all over the world. Three key challenges faced by the fluidic culture devices are affordability, transferability between multiple laboratories and robustness of the model. Here, we present the next generation of the Quasi Vivo® chamber system, the new Quasi Vivo® Advance Range, which we believe addresses the major challenges described above, particularly in developing air-liquid (skin, respiratory systems models) and liquid-liquid (intestinal, blood-brain-barrier, blood-retina barrier etc) interface models. The key features of our Quasi Vivo® Advance Range system are:

- **Portability and easy to use physiological flow system.** This feature allows independent controllable laminar flow of air, gas, or liquid to the apical and basolateral sides, allowing 9 to 12 culture models on a standard well plate dimension.
- **Compatibility with real-time microscopy.** The new Quasi Vivo® Advance Range comes equipped with optical windows on the top and bottom surfaces of the cell culture chamber ideal for high resolution imaging in real time.
- **Compatibility with most commercially available inserts and models.** Compatible with 3D, 2D, submerged, air-liquid or liquid-liquid interface models.
- **Compact multi-chamber and modular tray format.** Ideal for all-in-one multi-tissue/multi-organ modelling, with a small footprint to save incubator space.
- **No non-specific binding.** This new system is made up of inert and biocompatible resin and does not use Polydimethylsiloxane (PDMS) hence is expected to be reliable for pharmacokinetic studies.
- **Affordability and easy integration with standard laboratory equipment.** Quasi Vivo® Advance Range is inexpensive unlike most other commercial Organ-on-a-Chip systems and is functional inside the standard laboratory incubators and allow real time fluid sample collection. Access to the biological tissue is possible both during and at the end of experiments.

Session 3 - IMMUNOLOGY & ONCOLOGY MODELS

12. *In vitro* Immune niches for T cell development (Keynote lecture)

Øyvind Halaas, Norwegian University of Science and Technology, Norway

13. Metabolic-imaging of human glioblastoma live tumors: a new precision-medicine approach to predict tumor treatment response early

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Background: Glioblastoma (GB) is the most severe form of brain cancer, with a 12-15 month median survival. Surgical resection, temozolomide (TMZ) treatment, and radiotherapy remain the primary therapeutic options for GB, and no new therapies have been introduced in recent years. This therapeutic standstill is primarily due to preclinical approaches that do not fully respect the complexity of GB cell biology and fail to test efficiently anti-cancer treatments. Therefore, better treatment screening approaches are needed. In this study, we have developed a novel functional precision medicine approach to test the response to anticancer treatments in organoids derived from the resected tumors of glioblastoma patients.
Methods: GB organoids were grown for a short period of time to prevent any genetic and morphological evolution and divergence from the tumor of origin. We chose metabolic imaging by NAD(P)H fluorescence lifetime imaging microscopy (FLIM) to predict early and non-invasively ex-vivo anti-cancer treatment responses of GB organoids. TMZ was used as the benchmark drug to validate the approach. Whole-transcriptome and whole-exome analyses were performed to characterize tumor cases stratification.
Results: Our functional precision medicine approach was completed within one week after surgery and two groups of TMZ Responder and Non-Responder tumors were identified. FLIM-based metabolic tumor stratification was well reflected at the molecular level, confirming the validity of our approach, highlighting also new target genes associated with TMZ treatment and identifying a new 17-gene molecular signature associated with survival. The number of MGMT gene promoter methylated tumors was higher in the responsive group, as expected, however, some non-methylated tumor cases turned out to be nevertheless responsive to TMZ, suggesting that our procedure could be synergistic with the classical MGMT methylation biomarker.
Conclusions: For the first time, FLIM-based metabolic imaging was used on live glioblastoma organoids. Unlike other approaches, ex-vivo patient-tailored drug response is performed at an early stage of tumor culturing with no animal involvement and with minimal tampering with the original tumor cytoarchitecture. This functional precision medicine approach can be exploited in a range of clinical and laboratory settings to improve the clinical management of GB patients and implemented on other cancers as well.
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14. Brca2 breast organoids activate a luminal progenitor-like program following genotoxic stress
Mona Shehata, University of Cambridge, UK

15. Modeling skin inflammation using human 2D and 3D in vitro models
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Skin inflammation can be due to a variety of factors, including immune system dysfunction, allergic reaction, infections, photosensitivity, and injury or wound. Atopic dermatitis (AD) is a typical chronic inflammatory disease of the skin that affects children and adults around the world. Topical corticosteroids are first-line topical therapies for eczema. However, side effects can sometimes occur after chronic use. Natural compounds with anti-inflammatory properties are now commonly being incorporated into skin care products to combats skin inflammation. The objective of the study was to validate an in vitro assay using 2D and 3D skin models to evaluate anti-inflammatory properties of new drugs.
Normal human epidermal keratinocytes (NHEK) were irradiated with UVB, or treated with lipopolysaccharide (LPS), or cytokine cocktails IL-4 and IL-13 interleukins (AD model). Dexamethasone (DEX) and Pyridone 6 (JAK kinase inhibitor 1) were used as anti-inflammatory positive controls in keratinocytes treated with LPS and cytokine cocktail, respectively. 3D

reconstructed human epidermis (RHE) tissues were treated with LPS alone, or in combination with systemic treatment of DEX or topical treatment of Betamethasone cream. Untreated samples were used as control.
mRNA expression of TNF-alpha, CXCL8 (IL-8), filaggrin (FLG) and involucrin (IVL) in keratinocytes and RHE tissues was measured by quantitative real time RT-qPCR. Furthermore, concentration of IL-8 secreted in culture medium was measured by Enzyme Linked Immuno Sorbent Assay (ELISA).
In NHEK, UVB irradiation, LPS and cytokine cocktails induced significant increase of secreted IL-8, while DEX and JAK inhibitor significantly decreased induced production IL-8. In addition, cytokine cocktails downregulated the expression of both filaggrin and involucrin, while JAK inhibitor reversed this effect.
In RHE model, LPS and cytokine cocktails induced significant increase of IL-8 and TNF-alpha mRNA expression and secretion. Betamethasone cream and JAK inhibitor significantly decreased TNF-alpha and IL-8 mRNA expression and secretion.
The human in vitro skin models mimicking skin inflammation developed in the study will be valuable in assessing the efficacy and toxicity of new drugs.

16. **Effects of excipients on the absorption of drugs in the GI tract**
    Chloe Spencer, Sheffield Hallam University, Sheffield, UK

17. **Mass Spectrometry Imaging of 3D Tissue Models in Drug Development**
    Malcolm Clench, Sheffield Hallam University, Sheffield, UK

18. **PDMS-free gut on a chip as a tool for human microbiota derived extracellular vesicle research**
    Roberts Rimsa[2,3], Valerija Movcana[1], Karina Narbute[1], Janis Plume[1], Karlis Grindulis[2], Arnita Spule[3], Felikss Rumnieks[1], Vadims Parfejevs[3], Gatis Mozolevskis[2,3], **Arturs Abols**[1,3,*]

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    The gut microbiota has a critical role in human health and are involved in many if not all physiological and pathological processes, while bacterial derived extracellular vesicles (BEV) play a significant role in it, since they can transfer microbiota derived signal molecules. Currently, research of human gut microbiota communication mechanisms with host cells is complicated and research methods for these processes are limited. One of the most promising model systems to study these processes is the gut-on-chip (GoC) platform. Therefore, the aim of our research is to study cancer patient microbiota derived BEV RNA content, which can enter from gut lumen to circulation by applying GoC devices. This approach could be used as a potential biomarker identification tool for dysbiosis in future. To that end, we have currently developed a GoC device suitable for anaerobic microbiota cultivation and successfully optimised anaerobic microbiota isolation from human stool samples. Next, we cultivated cancer patient microbiota within GoC functionalised with stable cell lines for 72h and performed metagenome analysis, followed by epithelial/endothelial barrier integrity analysis. Results showed that after 72h, most bacteria are still strictly anaerobic or aerobic, however, the top 20 most common bacterial strains were changed depending on the patient after cultivation suggesting for selective pressure from media. Finally, BEV from the gut lumen and those that can pass through the gut-endothelial barrier were collected, analysed by NTA and currently we are analysing BEV RNA content.

Session 4 - 3D PRINTING / 3D PAINTING FOR IN VITRO MODELS

19. **3D bioprinting of tissue models: Challenges and potential (Keynote lecture)**
    Yeong Wai Yee, HP-NTU Corporate 3D Printing Centre, Singapore

20. **Building personalised gum tissue models using 3D bioprinting**
    DAI Yichen(1,#), LU Yuyun(2), LU Wen Feng(3,4), CHEW Jacob Ren Jie(1,5), HUANG Dejian(2), SRIRAM Gopu(1,4,*)

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Gingival recessions affect 50-90% of adult population [1] and 78% of lesions will progress with increased attachment loss and risk for root caries [2]. The gold-standard treatment using autologous gingival grafts is associated with significant donor site morbidity and limited graft availability [3] while alternatives including collagen-based matrices of animal origin have inferior clinical performance [4] and limited patient-specific modifications. Therefore, there is a need to develop non-invasive alternatives with good clinical efficacy and tailoring potential.
3D bioprinting has been increasingly employed for constructing tissue models with spatially controlled architecture but developing optimal printing protocols using bioinks with optimal rheology, biocompatibility and biofunctionality remain as major challenges. Hence, we aimed to 3D bioprint personalized models that morphologically and biofunctionally resemble human gum tissues for gingival regeneration.
Bioinks were developed by mixing polysaccharide-based thickeners (maltodextrin, xanthan gum and nanocellulose) with human fibrinogen. Rheological tests showed low loss factors (0.15-0.25) and decreased viscosity with increasing shear rate, indicating gel-like behaviour and shear-thinning behaviour of all bioinks. Post-print and post-culture (24h) pore printability indexes (0.85-0.95) demonstrated good shape fidelity. Swelling tests showed 2-20% pore area reduction within first hour in media with no further reduction over 24h period. AlamarBlue and Live-Dead assays demonstrated high cell viability (85-95%). After optimizing bioprinting parameters, 3D custom gum designs traced from clinical models were bioprinted and cultured under air-liquid interface. Histology and immunostaining showed formation of stratified squamous gingival epithelium over a mucosal matrix with vimentin-positive gingival fibroblasts. Immunostaining also confirmed expression of various keratinocyte differentiation and basement membrane markers, suggesting formation of full-thickness gingiva-like tissues.
In summary, we demonstrated 3D bioprinting of personalized gum tissue models using bioinks with good printability, cell viability and biofunctionality. Future work on in vivo engraftment of bioprinted gum tissues will help to understand clinical translational potential for regenerative periodontal therapy.
References: 1.Kassab MM, Cohen RE. J Am Dent Assoc. 2003 Feb;134(2):220-5. doi: 10.14219/jada.archive.2003.0137. PMID: 12636127.
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21. Applicability of the 'Cloudbuster' software tool for assessment of data generated in 3D environments

Sally Prior, Arndt Rohwedder, Sabine Knipp, **Anke Brüning-Richardson**

Introduction: Preclinical cancer studies currently include confirmation of effectiveness of novel treatments in in vivo models. In brain tumour research this involves the intracranial injection of rodents (mice and rats) with cancer cells to induce tumour formation. Although there has been a steady increase over recent years in the development of alternative, animal free, in vitro models, data generated from such assays are difficult to assess for the systems to be adopted for routine pre-clinical treatment screens. We have recently reported on a newly developed software, Cloudbuster, which allows assessment of pre-clinical data generated in 3D environments. Here confirm the applicability of the software to data generated by confocal microscopy, immunohistochemistry and MRI scans. Methods: Spheroids were generated from established glioma cell lines which were exposed to previously characterised inhibitors and then prepared for confocal microscopy and immunohistochemistry using our established methodologies. Anecdotal MRI scans from mice

intracranially injected with cells carrying knockdowns of identified pro- or anti-migratory genes were used for further analysis to compare results generated from spheroids from the same knockdown cells. Generated data were analysed using 'Cloudbuster', investigated parameters included tumour size/volume, protrusion length and number, and number of single detached cells. Results: 'Cloudbuster' performed efficiently to determine migration association parameters in all scenarios. These included volume and size of spheroids or tumours, protrusions away from original spheroids or tumours and number of single cells in the vicinity of the spheroids. The comparison of results obtained from MRI scans of the tumour carrying gene knockdowns and spheroids generated from the same cells revealed that there were striking similarities between the generated data. Conclusion: We followed up our initial studies with 'Cloudbuster' to determine applicability in view of integration of pre-clinical in vitro assays to ultimately replace in vivo models in cancer research. The data presented indicate that 'Cloudbuster' can be applied to data obtained by various means, eg, confocal microscopy or immunohistochemistry, and that data generated in 3D is comparable to data generated from in vivo MRI scans. This supports the use of alternative, animal free assays, for pre-clinical cancer studies.

22. Animal-free peptide hydrogels as a tool for novel cancer research

Dammy Olayanju, Manchester Biogel, UK

23. Developing a 3D chronic wound model using animal-free products.

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Chronic wounds are scenarios where the acute wound healing response is impaired, inducing a burden to both the patient and the healthcare system. In order to replicate the human wound repair process, animal models are often used, to provide a greater wealth of information on the wound healing potential of novel therapies. These models are typically rodent models, including the diabetic mouse model, demonstrating chronic wound healing scenarios1. However, these rodent models have limitations in their translation to human wound healing scenarios due to differences in healing manner. An alternative to animal models involves the use of 3D organotypic in vitro models, representing more closely the complex in vivo scenario the widely performed 2D monolayer cultures.
This 3D organotypic model comprises of human chronic wound derived fibroblasts and epidermal keratinocytes, cultured at the air-liquid interface, resulting in the differentiation of the various epidermal layers, including the protective cornified layer2. This 3D organotypic model is cultured over 14 days, before subsequent H&E staining, along with immunostaining for key components of the epidermal and dermal layers, including keratin 10, keratin 14, involucrin and fibronectin. The majority of cell-based research utilises animal-derived products, whereas these models are cultured using synthetic, animal-free products, resulting in a better consistency in the studies, due to the batch variability associated with animal product use3. Many industries and funders are already expressing an interest in replacing animal use in research, but a viable option is required to sufficiently replace these models. This 3D model will be a more cost-effective option than animal models and without the ethical considerations associated with their use.
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24. Gingival Crevice-on-Chip: Recapitulating Host-Oral Microbiome Interactions in Gum Health and Disease

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Objective: Accelerating the development of therapeutics necessitates advancing and improving the existing in vitro models to study gum tissue health and diseases. The microenvironment of the gum tissues (gingiva) around a tooth is unique as its connective tissue contributes towards constant flow of interstitial fluid termed as gingival crevicular fluid (GCF) which renders host protection. Towards this pursuit, we describe an Organ-on-Chip (OoC) system that mimics the cellular, structural, fluid transport and pathophysiological properties of the gingiva along with crucial microflora which tries to capture in vivo like innate immune responses.

Methods: The designed OoC device comprised of a central compartment housing the Gingival Connective Tissue Equivalent (G-CTE) and two adjacent fluidic channels one each for microbiome and flow of media respectively. Simulation of pressure and velocity profiles across the culture chamber was performed. G-CTE was cultured on chip for 9 days under interstitial flow and its interaction with oral microbes was studied. Long term co-culture with oral biofilm symbiont was performed on chip and the impact of simulated GCF (s-GCF) on the innate immune response was studied by compartmental interaction between oral biofilm pathogens and G-CTE.
Results: The OoC device design aided in the long term culture of G-CTE and recapitulated the functional features of Gingival Connective Tissue microenvironment. The device allowed primary microbial colonization by oral commensal bacteria and long-term existence of the host as well as its clearance secondary to s-GCF. The impact of s-GCF flow on the innate immune response by CTE upon exposure with endotoxin bearing oral microbe showed a reduction in the secretion of IL6, IL8 and CCL2 suggesting host protection and activation.
Conclusion: OoC system incorporated minimal set of structures and features necessary to recapitulate key biological features of host-oral microbiome interaction in vivo and offers capabilities which are an improvement over existing in vitro models.
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25. Microfluidic systems to understand host-material interactions in dental and oral-care

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Objective: Over the last decade, tissue fabrication technologies have progressed rapidly. Organ-on-a-chip systems and 3D organotypic cultures provide opportunities for biomimicry of 3D tissue architecture, cell-cell and cell-matrix interactions, miniaturization and controlled delivery/collection of nutrients, drugs, microbes and metabolites. However, the potential of these technologies for dental and craniofacial tissue regenerative applications are poorly explored.
Here, we present our recent efforts in the use of microfluidic organ-on-chip technologies to biofabricate tooth-on-a-chip and oral mucosa-on-a-chip for advanced assessment of dental and oral-care products.
Methods: Tooth-on-a-chip devices was fabricated by thermally bonding microstructured layers of poly(methyl methacrylate) (PMMA) sheets. The chambers and microchannels within each PMMA sheet was microfabricated by micromilling. Tooth slices of different thickness was used as representative of dentin barrier.Similarly, the multi-chambered oral mucosa-on-chip was fabricated within the RevIn™ chip (kindly provided by Revivo BioSystems, Singapore). Tissue-engineered full-thickness oral mucosal constructs were fabricated using a fibrin-based matrix within the multi-chambered microfluidic device.
Results: The tooth-on-a-chip device seeded with dental pulp stem cells (DPSCs) and fitted with dentin slices of different thickness was used to evaluate the barrier potential of dentin slices and toxicity to dental materials (silver diamine fluoride-SDF). Data showed that SDF penetrated the dentin slices (<1mm) and induced significant death of DPSCs. This data suggests the potential toxicity of SDF to dental pulp in deep cavity preparations. Oral mucosal constructs fabricated within the RevIn™ microfluidic chip showed a multi-layered squamous epithelium over a cellular lamina propria. The mucosal epithelium of the oral mucosal-on-chip was thicker compared to those cultured under static conditions in inserts. The oral mucosa-on-chip demonstrated the expression of stratified epithelial cytokeratins, matrix proteins and barrier proteins. Dental anaesthetic permeation studies demonstrated the barrier function of the oral mucosa constructs. The microfluidic design was utilised to flow through oral-care products (mouthwash) over the oral mucosal constructs with forward and backward flow to mimic the rinsing. Under flow conditions, data showed differences in the mucosal irritation potential of alcohol-free and alcohol-based mouthwashes. Conclusion: Microfluidic organ-on-a-chip technology provides a miniaturized platform to recreate microphysiological conditions

representative of the dental and oral mucosal tissues which can be used as versatile and physiologically relevant alternative to human and animal models.

26. Using Quasi Vivo millifluidics to investigate IPAD in clearance in the brain
Jennifer Dewing, University of Southampton, UK

27. In vitro Drug-Disease Models of Glioblastoma with Graded Complexity
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Glioblastomas (GBM) account for poor prognosis and dismal survival rates in patients due to their highly aggressive infiltrative nature to rapidly migrate within the brain. Experimental treatments for GBMs using animal models often elicit severe side effects and there are major doubts regarding the usefulness of such in vivo models when undergoing animal to human clinical translation. Here, we describe the development of a series of 3D bioengineered GBM models towards the generation of a more physiologically representative system in which candidate drugs can be tested. Glioma cell lines U87 and U251 derived from human GBM were used along with associated knockdowns of previously described anti-migratory and pro-migratory genes or anti-migratory inhibitors to examine their roles in the actin polymerization pathway in cancer cell migration. GBM models were fabricated by implantation of spheroids within hydrogel microenvironments that were fashioned to exhibit migratory collagen tracts within a non-cell adhesive outer shell. The distribution of collagen was organised to have low, intermediate, and high-density regions within the construct, replicating the complexity of native GBM. 3D models were then cultured under control conditions (media only) or treated with anti-migratory drugs (CCG-1423, rhosin or combination). Using light-sheet and confocal microscopy, migration velocity, cell phenotype and migratory behaviours were analysed in live and fixed tumour mimics. In models where migration is promoted, actin was vastly upregulated and cells assumed a migratory mesenchymal phenotype, whereas under anti-migratory drug treatment, cells were amoeboid in shape with a dramatic reduction in actin expression and consequently limited migration velocity. Here, we have demonstrated that it is possible to develop biologically-relevant GBM models that capture the anisotropic nature of the tumour microenvironment using multi-layer biopolymer engineering. The ultimate goal of this research is to develop technology that can help provide personalised treatments for GBM and subsequently improve patient outcomes.

28. Membrane technology for tissue engineering
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The incorporation of membranes into tissue engineering products has grown significantly. One example is the use of microporous TRAKETCH membranes in systems like organ-on-a-chip and cell culture inserts. Tissue-culture treated membranes are excellent supports for cell growth [1]. To select the best membrane for tissue engineering it is important to consider the fabrication method, material, porosity and surface modifications [2]. In this work we would like to shed light on the technology and science behind microporous plastic membranes.
TRAKETCH membranes are produced from ultra-thin PET films, that are bombarded with accelerated noble gas ions. The goal is to break the molecular chains of the polymer to create ion tracks that are clearly defined by their density and angles. The desired pore density is accurately determined by the ion beam intensity and the film velocity. The precise control of these factors allows the production of superior optical clear membranes. Afterwards, the ion tracks are chemically etched into pore channels. The diameter of these pores can be determined with sub-micrometer accuracy. Finally, membranes are treated with air plasma to promote optimal cell attachment.
TRAKETCH membranes are produced in-house by SABEU, guaranteeing 100% parameter consistency and are integrated in our line of cell culture inserts cellQART [3] and in recent

innovative organ-on-a-chip platforms supporting the 3D growth of human mature adipose tissue [4] and the creation of an oviduct-on-a-chip [5].
Cells grown on microporous membranes in cell culture inserts, have access to nutrients from the apical and basolateral side. This allows the separation into two compartments, simulating the physiological condition where the cell layer acts as a diffusion barrier. Due to the physiological relevance of this model, it is possible to mimic the complexity of human relevant systems, co-cultures and tissue barriers [6,7], such as the epithelium or endothelium [8].
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29. High-throughput renal organoids: a novel use for NaviPlate technology

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The area of renal disease modelling is lagging behind many other tissue areas of research due to the enormous complexity of this organ. Over the past decade, several robust protocols enabled small scale generation of renal organoids from induced pluripotent stem cells (iPSCs). Our innovative technology, NaviPlate, is aiming to expand the utility of complex biology in early drug discovery, especially in those steps involving high-throughput screening (HTS) and automation. In this conference, we will reveal how we developed several service offerings based on NaviPlate to serve drug development. These services are based on an industry standard 384-well NaviPlate. Our most important objective in creating NaviPlate was to enable the use of complex biology models in discovery biology, especially those involving high-throughput drug discovery. The challenge was double: to provide a technology that is industry-ready, i.e., 384-well SBS format and to make it flexible enough to accommodate a range of complex biology assays. The physically small size of each well aimed to perform assays with lowest number of cells.
Here, we will give an overview of how we took existing iPSC-derived renal organoid protocols and used the NaviPlate to generate a high-throughput renal disease models. Our NaviPlate-based solution generates highly reproducible organoids in 30 days with finely tuned size control. These organoids require ~10-fold less cells than those required in published protocols. The organoids display typical kidney architecture, including LTL+ proximal tubules and E-cadherin+ distal tubules. Finally, our efforts to build on these normal organoids to develop accurate models of human kidney disease will be discussed. A special emphasis will be given to the challenges of combining these efforts with HTS format, required for drug discovery in academia and industry.
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30. 3D Liver Microtissues - Translational Drug Development Tools for DILI (Keynote lecture)

Armin Wolf, Insphero AG, Switzerland

31. Developing 3D Peripheral Nerve Injury models

Caroline Taylor, University of Sheffield, UK

32. A Morphological Micro Scale Model of Scar Tissues of Hip Capsule Ligaments Grown Around Different Implant Materials

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Arthroplasty is the surgical procedure that aims to replace the hip joint by artificial materials. The fibrous tissue that reforms around the hip joint after the surgery plays an important role in joint stability. In this study, the morphological and mechanical properties of the scar tissues that form around implants composed of either polymer and metal or ceramic are compared to native tissue removed during an initial total hip arthroplasty. Immuno-histological analyses of the samples revealed different hierarchical structures of the tissues over three scales: the fiber, the fascicle and the tissue scales. At the tissue scale, micro-tensile tests were performed on millimetric tissue samples. First and second Piola-Krichhoff stresses and normal and Green-Lagrange strains were calculated to build the constitutive laws at the macro and then micro scales. The tissue non-linear elastic responses were identified by either an exponential law or an Ogden third-order constitutive model. At the fiber scale, a patient-specific micro-scale finite element model for a single fiber as well as for multiple fibers was created based on measured morphological parameters such as the amplitude and period of the fibers. The FEM unit cell includes the different material parameters for each tissue type and distinct Ogden constitutive models for the fiber and the matrix composed of a mixture of fibers in ground substance. Three regimes of loading were determined at the tissue scale and applied at the microscale model. The microscopic stress and strain fields were calculated for the three different FEM models for the different tissue types and then they were compared to the experimental curves in the macroscale. The results indicate differences between the mechanical and morphological properties of the tissues that have formed around different implant materials.
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33. A Bone-on-Chip as a 3D in Vitro Platform to Assess the Effect of Stem Cell Age on Bone Quality and Test Medicines for Bone related Pathologies

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We present a physiologically relevant 3D environment to form human bone and disk organoïds from decellularized bones and either stem cells of different ages or primary committed cells on which regenerative treatments could be tested.
Our bone-on-chip includes decellularized bones that are recellularized with either human primary adult Mesenchymal Stem Cells (MSCs), fetal human osteoblast progenitors (FHOB) or human primary annulus fibrosis (hAFC) and nucleus pulposus cells (hNPC). MSCs and FHOBs differentiated into stem cell derived osteocytes (SCDOs) within 30 days and formed bone. AFCs and NPCs formed a collagenous matrix. The systems were long-term cultures up to 26.5 months. Compressive mechanical stimulations applied to the constructs mimicked human walk.
Early and mature osteocytes were identified by the secretion of E11 and SOST under fluorescent confocal microscopy. The cells formed mineralized collagen layers of alternating orientations that created an extracellular matrix able to assemble multiple bones revealing topological cues sensed by the cells within minutes of seeding. The stiffnesses measured using 3-point bending showed primary adult MSCs formed a volume of new tissue that was half of the initial bone volume of mineralized tissue and that displayed a flexural stiffness of 13 Mpa, equal to a fifth of the 69 MPa stiffness displayed by the decellularized bone and new bone mix at 109 days. Fetal cells formed smaller quantities of highly mineralized bone of stiffness of 93 MPa at 126 days. The stress fields around the cells were quantified using numerical twins of the cells and tissues indicating pico-Newton forces. FTIR measurements confirmed that Fetal cells produced highly mineralized bone

that contained also lipids, while adult cells assembled multiple bones to recreate the Haversian structure.
The new results confirmed the influence of the age of stem cell on their ability to produce higher quality bone when from younger individuals.
The authors thank NSF CMMI BMMB 1214816 EAGER, CNRS INSIS PEPS Mecanique du future, the Farman Institute, Synchrotron 20191708 and 20201633 awards, the Anatomical Gift Association of Illinois.
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34. Development of an evolutionary algorithm for identifying design criteria for in vitro constructs

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Natural selection is the process through which populations of living organisms adapt and change. This process can be simulated as an optimization problem via genetic algorithms [1-2]. Here, we exploit genetic algorithms to identify design criteria for in vitro constructs. Our aim is to generate virtual models able to select the most appropriate size and shape of cell aggregates and, in the future, even replace in vitro models.
A starting population of in silico models of 3D cell aggregates with random characteristics in terms of dimension and shape was created. The population is tested in terms of a fitness function (FF): a mathematical formula quantifying how an individual in the population is suitable or not. In this preliminary study, the fitness function considers the minimum oxygen concentration within the aggregate and its sphericity (i.e., how close the actual surface is to a perfect spherical surface, so minimizing surface energy).
Firstly, all individuals are assigned with a score, consisting of the computed value of the FF. Then, individuals with best scores are selected automatically to populate the next generation. Based on a parallelism with genetic processes in biology crossover and mutation are performed to generate the remaining offspring of the next population. These steps are repeated until the best combination of genes (i.e., giving the best score) comes out [3]. To our purpose, this optimal individual represents the cell aggregate with optimal morphological and dimensional characteristics.
Preliminary results suggest the feasibility of our approach. In fact, some of the optimal individuals hold the required characteristics (i.e., minimum oxygen concentration ≥ 0.04 mol/m^3). Future developments will include the definition of a more reliable FF, accounting for further features (i.e., cell-cell and cell-environment interaction). More sophisticated dynamics of crossover and mutation will be also implemented in order to better mimic biological counterparts.
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35. An imaging pipeline for quantifying cellular morphology and arrangement

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All living organisms are constantly changing shape. In in-vitro systems such as organoids, spheroids or other three dimensional constructs, it is well known that cellular morphology gives important insights on cell-specific functions or responses to external stimuli from the environment.
Many computational frameworks include quantitative image analysis to study how tumor spheroid growth, organogenesis and toxicity impact on their morphology [1, 2, 3]. Moreover, cellular organization and arrangement is crucial for determining tissue function, e.g., in terms of cell-cell interaction and behaviour [4, 5]. These features can be accurately quantified in-vitro and enable a better understanding of the physical extent of cell-cell communication in living as well as engineered tissues.

To this end, we developed an imaging pipeline to identify a cell compactness index, called Proximity Index (PI), and evaluate cell sphericity in constructs of different dimensions (i.e., 2D and 3D) and densities. Briefly, confocal stacks are segmented to highlight cell nuclei; then, for each nucleus, both centroid coordinates and sphericity [6] are estimated. Then, the number of intersections at various distances from each cell body was recorded [7] and the PI was estimated as the inverse of the average cell-cell distance, weighted by the corresponding number of intersections.
For testing the pipeline, HepG2 cells were cultured in both monolayers and alginate-based spheroids [8] at different cell densities, stained with DAPI and phalloidin and acquired using a confocal microscope.
The PI is able to return information about cell compactness that can be also compared among construct dimensions and densities. Regarding the sphericity, cells in monolayers tend to spread onto the culture substrate, while those arranged in 3D configurations are characterized by a round shape, and thus can be more densely packed. The pipeline implemented allows quantifying the physical extent of cell-cell interaction and gives crucial insights for developing computational models with enhanced predictive potential.
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Session 6 - EMERGING TECHNOLOGIES FOR ANIMAL REPLACEMENT/ ANIMAL FREE MEDIA REPLACEMENT

36. **Animal free technologies (Keynote lecture)**
    Carol Treasure, XCellR8, UK

37. **Biomaterials for animal replacement**
    Ipsita Roy, University of Sheffield, UK

38. **Replicating human pharmacokinetics with a microphysiological system for preclinical studies in vitro**
    Dharaminder Singh, CN-Bio Ltd, UK

39. **Customisable peptide hydrogels as user-defined biomimetic models of specific tissues**
    Johnathan Curd1*, Jennifer Ashworth1, Christopher Merrett2, Neil R. Thomas2, Alan Mcintyre1, Cathy Merry1
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    2 School of Chemistry, Biodiscovery Institute, University of Nottingham UK

    The extracellular matrix (ECM) is comprised of a complex variety of biochemically distinct molecules including proteins, glycoproteins, proteoglycans, and polysaccharides. These provide not only the physical support for resident cells but also play a vital role in tissue homeostasis. However, current *in vitro* models of development and disease typically fail to recapitulate the complexities of the ECM without relying on animal-derived products, leading to a poor extrapolation of in vitro findings to in vivo relevance. This has driven the recent interest in the development of more sophisticated synthetic models which allow customisation of matrix composition and stiffness.
    Here we present a new candidate model platform based on the self-assembling gelator peptide FEFEFKFK which allows end users to fine-tune both its mechanical and chemical properties using a variety of available methods. Its stiffness can be controlled independent of its composition, allowing end users the ability to investigate the biological impact of matrix additions independently from tissue mechanics. Soluble matrix components selected to match specific tissues can also be encapsulated, to drive cell behaviours in specific ways. Two new methods for covalent immobilisation of matrix components are also presented, utilising either enzymatic or “click” chemistry reactions.
    The available methods for customisation now allow end users more flexibility in how they approach model design and can be combined to create more complex, and faithful, models of specific tissues. Furthermore, the new techniques for customisation are compatible with similar peptide-based models. These models present researchers with the opportunity to study the underlying

mechanisms of development and disease in an environment which produces more reproducible and human-relevant data. The potential impact of this will be to help reduce the number of animals in research, to help reveal new drug targets previously obscured in less accurate models, and to better stratify those new drugs in preclinical studies.

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