SESSION POSTER

1

Sophie Langouet (Inserm IRSET UMR1085) Characterization of 3D model of human liver organoids encapsulated in microbeads

3D culture of human spheroids and organoids offers numerous advantages in terms of hepatocyte differentiation, long-term culture and multicellular structuring closer to the human liver. Our Hepoid® model enables human hepatocytes to be organized into small, hollow spheroids that are polarized, proliferating and highly differentiated over several weeks. We have integrated a dynamic component using innovative microfluidic technology, to obtain Hepoid® encapsulated in methacrylated gelatin microbeads containing human hepatocytes (HepaRG) and stellate cells (LX2), alone or in co-culture. Our results show reliable and reproducible production of Hepoid® encapsulated in microbeads, rapid spheroid/organoid formation in the microbeads, increased cell viability as a function of culture time for up to 30 days, and highly differentiated hepatocyte function.Moreover, these microbeads support continuous agitation, compatible with their use in a bioreactor. Our 3D microbead-based model provides a robust model for human liver organoid culture, with promising prospects for toxicology and the study of liver fibrosis.

2

Charlotte Rivière (ILM / CRCL) Integrating 3D Tumor Models and Hydrogel-based Microfluidics for Metabolic Control.

Disordered angiogenesis is a hallmark of cancer, leading to poor tumor perfusion and the formation of gradients in oxygen, pH, and nutrients, affecting therapeutic response. In tumor tissues, nutrient gradients progressively develop as a direct consequence of tumor growth. However, current 3D cell culture models fail to accurately replicate these conditions, hindering accurate assessment of cancer cell behavior. Instead, these models often rely on abrupt changes in culture media with decreasing nutrient concentrations, which poorly mimic the physiological environment of tumor tissues. To address this limitation, by combining numerical and experimental approach, we developed a hydrogel-based microfluidic system to precisely control the spatial and temporal distribution of metabolic supply to 3D tumor models, mimicking the poorly vascularized tumor microenvironment. This system applies to spheroids, and patient derived organoids, enabling in-depth understanding of cell responses within pathological context. As a proof of concept, we focused on L-glutamine metabolism and demonstrated the effectiveness of our system in studying cellular responses to their metabolic environment.

3

Maria José Perez Jimenez (Institut Imagine) Microglial PITRM1 deficiency triggers senescence and neurodegeneration in human iPSC-derived Brain Organoids.

Mitochondrial homeostasis is essential for microglial function, and its disruption is increasingly implicated in neurodegenerative diseases. We developed a human iPSC-derived brain organoid model to investigate how microglial mitochondrial proteotoxic stress contributes to brain pathology. Using microglia lacking the mitochondrial protease PITRM1—mutated in a rare neurodegenerative disorder with Alzheimer-like features—we modeled chronic mitochondrial stress. When integrated into wild-type brain organoids, PITRM1-KO microglia induced non-cell-autonomous effects, including increased P21 expression, neuronal death, and protein accumulation after 35 days. These findings demonstrate that microglial mitochondrial dysfunction is sufficient to propagate senescence and neurodegeneration in human brain tissue. This model provides a mechanistic framework to study microglial contributions to brain aging and mitochondrial disease, and a platform for testing therapeutic interventions in a human-relevant 3D system.

4

Jean Cacheux (LAAS-CNRS) Pancreatic tumour explant-on-chip to optimize drug transport and biodistribution.

Pancreatic cancer has one of the poorest prognoses, largely due to late diagnosis and limited treatment options. Therapeutic success in vitro often fails to translate in vivo, in part because the tumor microenvironment (TME) impedes drug delivery through its dense, stiff stroma and low permeability. We hypothesize that these physical barriers are key contributors to treatment resistance. To explore this, we propose a tumor-on-a-chip model using patient-derived pancreatic tumor explants that preserve native tissue architecture and cellular heterogeneity. Our approach integrates PRESTO, a microfluidic poroelastometry technology, to precisely control and monitor the tissue's poromechanical properties. This platform will enable us to evaluate how physical constraints affect drug transport and biodistribution, using therapeutic agents ranging from small molecules to oncolytic viruses. Ultimately, this interdisciplinary model aims to map the mechanical barriers within the TME and guide the development of more effective, personalized delivery strategies.

5

Marie Frenea-Robin (Université Lyon 1, Laboratoire Ampère) Assessment of combined therapies for pancreatic cancer treatment based on different physical stimuli using a hydrogel-based 3D cell culture platform.

Pancreatic cancer is becoming one of the leading causes of cancer mortality and has a 5-year survival rate of 10%. This poor prognosis is linked to the presence of a particularly dense tumor microenvironment (MET) preventing chemotherapy from reaching its target. Various studies show that a modification of MET favoring tumor chemosensitivity can be obtained by application of physical stimuli. Here, we present a versatile hydrogel-based 3D cell culture platform and demonstrate its interest for studying the effect of different physical approaches such as electrochemotherapy (the combination of electric pulses application with the administration of a chemotherapeutic agent) or photothermal hyperthermia on pancreatic cell spheroids. Instrumentation of the platform is currently underway to enable multimodal characterization of spheroids before and after treatment. We are also developing a 3D co-culture model incorporating cancer-associated fibroblasts (CAFs), with the goal of assessing the impact of the extracellular matrix on treatment outcomes.

6

Pierre Gaudriault (Cherry Biotech) Generating real life like preclinical data with 3D organs on well: case studies on lung cancer, adipose tissue organoids, and vascular toxicity, is biological complexity accessible.

To solve the drug development productivity crisis, new 3D models like organoids, tissue equivalent, or multicellular 3D cell culture are foreseen as the most promising despite lacking compatibility with standards, throughput and key biological features (vascular interface and immunity). We have developed CubiX to solve these bottlenecks, and are illustrating its versatility with 3 use cases: 1) Human mature adipose tissue organoids: Dynamic perfusion (CubiX) in a standard multiwell plate (MWP) significantly improved maturation of adipocytes, viability of fibroblast, and differentiation of EC. 2) Lung cancer organoids: Dynamic perfusion (CubiX) in a MWP allow the maintenance of immunocompetent organoids, with resident macrophages, fibroblasts, tumour cells, and circulating monocytes. The use of an organ on well approach allow to use this model for pharmacokinetics studies. 3) Drug Induced Vascular Injuries: The CubiX system allow the precise control of the shear stress (from < 1 to >12 dyn/cm^2) inside each well of a standard multiwell plate, needed to obtained a proper vascular interface. This multiwell plate differentiated endothelium is suited for DIVI assessment of drug can

7

Hariam Raji (Institut Imagine, Université Paris Cité) A multi-organoid microfluidic approach to investigate the gut-brain axis in Parkinson’s disease.

Parkinson's disease (PD), the most common age-related degenerative movement disorder, involves progressive loss of dopaminergic neurons, leading to motor and non-motor symptoms. Gastrointestinal symptoms often precede motor impairment, suggesting disease onset in the gut. A hallmark of PD is the presence of alpha-synuclein (A-SYN) aggregates and inflammation in brain and gut. Suitable models replicating human PD and inter-organ interactions are lacking. This project aims to establish an organoid-based microfluidic system mimicking gut-brain communication to study proteinopathy and A-SYN propagation in PD. We developed midbrain organoids (hMOs) using a novel protocol enriching PD-relevant neuronal subtypes. Immunofluorescence and functional analyses revealed a significantly higher number of mature and more functional dopaminergic neurons. In parallel, intestinal cultures and parasympathetic neurons are being cultivated in microfluidic devices to be connected with hMOs. Interactions will be assessed by immunohistochemistry, biochemical analysis, and live imaging. This system will provide a physiologically relevant model to study the gut-brain axis and early-stage PD pathology.

8

Guillaume Perry (Sorbonne Université) Building a human glomerular filtration barrier-on-chip: molecular and cellular approaches.

The glomerular filtration barrier plays an essential role in maintaining the blood homeostasis and eliminating xenobiotics thanks to a trilayer structure composed of: (1) glomerular endothelial cell layer, (2) the glomerular basement membrane, a layer of extracellular matrice and (3) podocytes, specialized cells forming slit diaphragm. In this project, we aim to reconstitute this specific architecture within a microfluidic device. Firstly, we aim to reconstructing the glomerular basement membrane using molecular self-assembly and shaping the membrane the properties of laminar flows within microfluidic devices. We are developing the process to control the assembly and characterizing it on open surfaces before transfering the protocol into microfluidic device. In order to form both cellular layers, human induced pluripotent stem cells (hiPSCs) have been differentiated into endothelial cells and podocytes using established protocols. We have validated our protocol using different hiPSC lines to generate glomerular cells, which have been characterized at different differentiation stages. We are performing coculture and perfusion experiments within microfluidic device.

9

Federico Bertoli (Institut Imagine) Investigating early mechanisms of alpha-synuclein pathology in iPSC-derived midbrain organoids.

Early Parkinson's disease (PD) involves α-synuclein (α-syn) misfolding, but links to later dopaminergic (DA) neuron death are unclear due to inadequate models. Here, we developed induced pluripotent stem cell (iPSC)-derived midbrain organoids as a more advanced and accurate model to investigate the dynamics and mechanisms underlying early events in α-syn pathology in PD. Inducing α-syn aggregation with preformed fibrils (PFFs) replicated early pathology features: PFF uptake, lysosomal colocalization, and p-Ser129 α-syn. Single-cell RNA sequencing revealed PFF-associated changes in a vulnerable DA neuron subpopulation. These neurons showed downregulated oxidative phosphorylation (impaired energy) and upregulated protein degradation pathways (proteotoxic stress response). Notably, mitochondrial NMNAT3 was selectively downregulated in affected DA neurons, suggesting altered NAD^+ metabolism contributes to their susceptibility. This study validates midbrain organoids for modeling early PD pathology and provides new insights into mechanisms of DA neuron vulnerability, particularly involving NMNAT3 and mitochondrial NAD^+ metabolism.

10 **Aimé Carole (CNRS, Département de Chimie, Ecole Normale Supérieure) Cell sheet-based vascularized dermis on-a-chip.**

Significant advances in organs-on-chip call for models displaying more relevant microenvironments. While natural or synthetic biomaterials limit the structural and compositional complexity of the extracellular matrix (ECM) that is the basis for the development of the microenvironment, cell sheets rely on the ECM produced by tissue-specific cells (PMID: 37406716). Although their use in clinical regenerative medicine has progressed, transposing cell sheets into microfluidic devices remains a technological challenge. We have recently developed a thick vascularized dermis model (PMID: 39182802). We have here microfabricated devices for: -1/ the fusion and insertion of vascularized cell sheets into a microfluidic chip; and -2/ the perfusion of microvascular networks. These tools will enable perfusion of the microvasculature developed in the dermis substitute, thus solving major technological challenges for interfacing biomaterial-free thick tissues-on-chip. Its development towards vascularized full thickness skin will pave the way for clinical innovations for personalized pathological models, and industrial innovations for drug and cosmetics testing, reducing the use of animal models.

11 **Abdelkader Teibi (MABLab ULR4490, ULCO) Generation and characterization of bone marrow organoid-like (BMO-like).**

Cleft palates are the most common congenital malformations in France (1/700 births), often requiring early surgical intervention, including autologous bone grafts from the iliac crest. Tissue engineering has emerged as a promising alternative, potentially eliminating donor-site morbidity and enabling earlier cleft closure. While bone substitutes combined with osteoblast progenitor cells have shown promise, they still fall short of regenerating fully functional tissue. Organoids, 3D cellular models replicating tissue architecture and function, offer a new avenue. We developed a human bone marrow organoid (BMO-like) by fusing two spheroids: microvascularized osteoblastic spheroid and adipocytic spheroid (hMSCs differentiated into osteoblast or adipocyte). Optimal culture conditions were studied to maintain different cell types in spheroid and BMO-like. Growth, morphology, viability, and expression of specific markers (osteocalcin, PPARγ2, Plin1, CD31) were evaluated to determine the optimal culture conditions. Cellular distribution was analyzed by immunostaining. The next step will be to evaluate whether these BMO-like organoids replicate full tissue functionality.

12 **Cyril Cerveau (4Dcell) High-Throughput 3D In Vitro Assay for the Formation of Reproducible, Anchored Spheroids on a Single Focal Plane.**

Spheroid culture often suffers from size variability, poor reproducibility and loss during routine handling. Our high-throughput assay uses a PEG-based microstructured hydrogel containing 19 and 91 microwells per gel, each guiding rapid cell sedimentation and self-assembly into uniform spheroids within hours. A tiny contact point tethers each spheroid to the glass bottom, preventing loss during media exchanges and maintaining all organoids on a single focal plane for live, high-resolution imaging.

In mesenchymal stem cell tests, spheroid diameter scaled cubically with seeding density and showed only 1.94 % standard deviation after 10 days. Under exaggerated media exchanges, anchored gels retained over 88 % of spheroids versus 14 % for unanchored controls. The hydrogel's nutrient-permeable structure supports cultures up to six weeks. Compatible with standard 96-well and 24-well formats (≈5 280 or ≈2 184 spheroids per plate), and fully automation-ready, this plug-and-play assay delivers reproducible 3D models at scale, ideal for fundamental biology, high-content drug screening, toxicology and regenerative medicine studies.

13 **Agathe Subtil (CNRS / Institut Pasteur) FtOCUS: Fallopian tube on chip to understand and treat tubal dysfunctions.**

Fallopian tubes (FT) connect the ovaries to the uterus and thus play a pivotal role in reproduction. Tubal factor infertility (TFI) accounts for a large proportion of infertility diagnoses. Nonetheless, the physiology of the FT and its dysfunctions are poorly known, due to the lack of a suitable model. FtOCUS will develop an organ-on-chip model to advance the current understanding of human FT in physiological and pathological contexts, provide tools for translational approaches and initiate those. We will focus on two conditions driving FT dysfunctions, infection by Chlamydia trachomatis, the main infectious cause of TFI, and endometriosis. The FT-on-chip (FToC) model will integrate, under hormonal control, epithelial, mesenchymal and immune cells obtained from women undergoing tubal section (healthy and pathological samples). This complex model system will enable to understand the molecular and cellular signalling leading to cilia dysfunction and fibrosis as well as to test the effect of recurrent stresses, as occurring during endometriosis or chronic infections. We will also identify biomarkers of fibrotic behaviour and test drugs for their ability to prevent tubal dysfunctions.

14 **Bianca Ionela Slivinschi (Institut Imagine) A novel iPSC-derived GUT-on-chip model for studying Parkinson's Disease pathology.**

Parkinson's disease (PD) is now recognized as a multisystem disorder and the gut, in particular, plays a crucial role. However, investigating the gut-brain connection in PD is challenging due to the limitations of current models. Recently, organs-on-chip emerged as a promising alternative, enabling dynamic and compartmentalized culture conditions. In a collaborative study, we developed a gut-on-chip system with a cyclic mechanical stimulation to mimic peristalsis. We incorporated iPSC-derived intestinal organoids and immunofluorescence analysis revealed that mechanical stimulation enhances tissue maturation. Preliminary data demonstrated the feasibility of integrating iPSC-derived macrophages and enteric neurons (already integrated in 3D organoid models), adding a critical neuro-immune component. Further assays will reveal cell-specific responses to pro-inflammatory signals in a PD patients-specific genetic background. In conclusion, this dynamically stimulated, iPSC-derived gut-on-chip represents a promising platform for investigating PD-specific mechanisms in a physiologically relevant model, providing insights into the role of intestinal inflammation in the pathogenesis of PD.