A vascularized hepatocellular carcinoma-on-a-chip for studying angiogenesis in tumor progression

I. Scientific context

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer and has one of the lowest survival rates, around 20%, among all cancer types [1]. The tumor microenvironment (TME) plays a crucial role in liver cancer progression and metastasis by providing biochemical and biomechanical signals that regulate tumor cell behavior. **HCC is characterized by a highly vascularized TME**. It exhibits a notable predisposition toward vascular invasion, and its angiogenic activity is directly associated with the likelihood of vascular invasion and carries prognostic significance [2]. **Tumor angiogenesis**, the development of new blood vessels to the growing tumor, is a foundational hallmark of cancer, which significantly influences the progression of HCC. Hepatic tumor growth necessitates neovascularization, resulting in pronounced neoangiogenesis in HCC development, reinforcing its classification as a hypervascularized tumor [3]. Tumor expansion relies on angiogenesis, with the tumor vasculature serving vital roles in nutrient and oxygen supply and waste disposal in tumor tissue, and acting as a gatekeeper for tumor cell metastasis to distant organs. Abnormal angiogenesis in HCC leads to leaky, dysfunctional vasculature, promoting hypoxia, immune evasion, and metastasis [4]. **Since angiogenesis promotes tumor growth, cancer metastasis and therapeutic resistances, a deeper understanding of the mechanisms regulating physiological and pathological angiogenesis is crucial for developing more effective treatment strategies.**

Tumor angiogenesis is driven by a complex interplay of biochemical and biomechanical factors. While biochemical factors such as growth factors and cytokines have been widely studied, there is limited research on how flow-dependent mechanical stimuli (e.g., extracellular matrix (ECM) stiffness, blood flow shear stress, stretch, tissue interstitial flow) influence endothelial cell behavior and vascular remodeling in HCC. Increased ECM stiffness, often due to fibrosis, has been reported to significantly promote angiogenesis by activating endothelial cell signaling pathways that drive migration, sprouting, and vessel formation [5]. Studies indicate that low shear stress, associated with impaired flow, promotes endothelial dysfunction, vascular endothelial growth factor (VEGF) overexpression, and leaky vasculature [6]. Similarly, hepatic interstitial flow could modulate VEGF gradients, influencing endothelial sprouting and metastasis [6]. **These mechanical cues are believed to be among the earliest signals initiating tumor angiogenesis in HCC, though their mechanisms remain poorly described. Investigating the interaction between angiogenic signaling, endothelial cell behavior, and mechanical forces will provide key insights into HCC progression and lead to novel therapeutic strategies targeting tumor vasculature.**

Studying such mechanical forces *in vivo* presents challenges, highlighting the need for advanced *in vitro* HCC models that can replicate mechanotransduction. Advances in microfluidic organ-on-a-chip (OoC) technology offer precise control over critical parameters such as fluid dynamics and microscale geometry, allowing the incorporation of mechanical stimuli that closely mimic *in vivo* conditions. Early HCC-on-a-chip models co-cultured hepatocytes with endothelial cells (ECs) in multichannel systems, where ECs and controlled fluid flow simulated vascular dynamics [7]. Although this setup facilitated studies on EC-hepatocyte interactions and their response to mechanical and chemical signals, their 2D structure lacked the architectural complexity and the perfusion dynamics of 3D HCC tumors, which are essential for accurately replicating tumor physiology and microvascular function. Recent efforts have focused on constructing functional vasculature-on-a-chip by using ECM-based hydrogels as pro-angiogenic factors to promote EC self-organisation into vascular networks, mimicking angiogenesis [8, 9]. These engineered vascular beds have been integrated with 3D liver spheroids [10]. While various levels of 3D vascularization have been obtained, **achieving functional intravascular perfusion within**

liver tumor spheroids—a key requirement for replicating blood flow dynamics and nutrient exchange in the TME—remains an unresolved challenge, which limits their application in HCC research.

II. Objectives

This project focuses on developing a physiologically relevant, perfusable vascularized human HCC-on-a-chip model that accurately recapitulates key TME factors. This model is poised to bring new insights on how mechanical events influence HCC tumor angiogenesis, thereby affecting HCC progression. Particularly, it seeks to unravel the molecular mechanisms orchestrating these processes. By deciphering key mechanotransduction pathways that drive HCC progression, this project endeavors to deepen our understanding of HCC angiogenesis, and advance the development of vasculature-targeted therapeutic interventions.

III. Approaches

(i) Development of a perfusable vascularized human HCC spheroid-on-a-chip model (Fig. 1): Utilize a microfluidic device that has already been designed and fabricated in the team to integrate human HCC spheroids (e.g., from cancer cell lines and primary cells obtained from surgery) containing pre-developed internal vasculature into an engineered vascular bed. The goal is to establish functional anastomosis between the spheroids' internal vasculature and external vessel networks.

(ii) Investigation of mechanical cues in tumor angiogenesis: Assess the impact of mechanical cues, such as ECM stiffness, fluid shear stress, stretch, and interstitial flow, on endothelial permeability, vessel maturation, and function at both cellular and molecular levels. .

(iii) Investigation of tumor angiogenesis in cancer progression: Evaluate the effects of abnormal vascularization on tumor growth, progression, and metastasis potential through tumor-endothelial interactions.

(iv) Identification of molecular mediators orchestrating tumor angiogenesis and progression: Focus on key mechanotransduction signaling pathways in endothelial cells that regulate tumor progression and identify potential therapeutic targets.

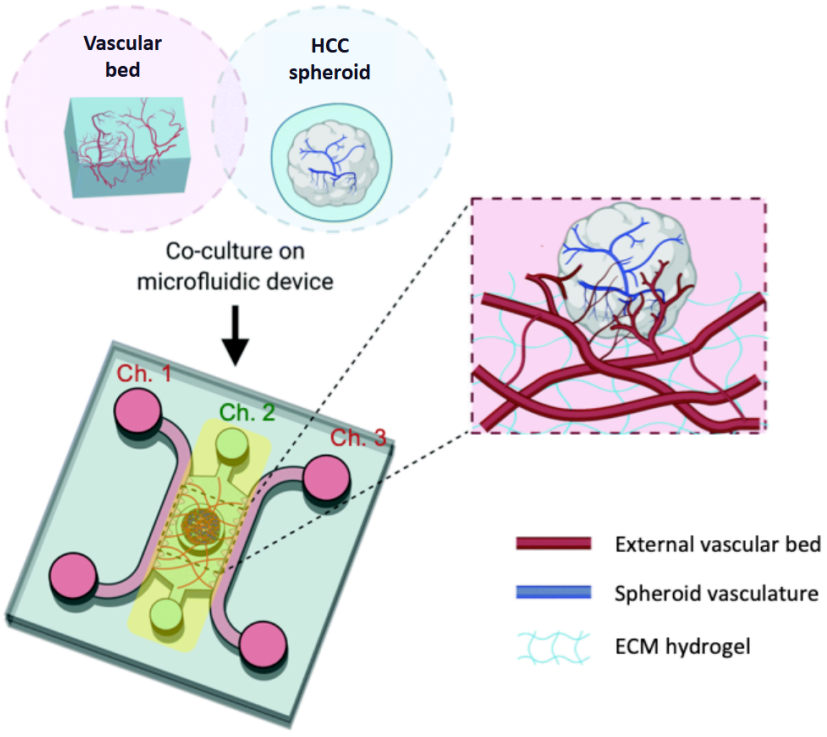

Fig. 1. Schematic representing the establishment of anastomosis connection between an external vascular bed (red) and pre-developed vasculature within a HCC spheroid (blue) inside a 3-channel microfluidic chip. (adapted from [11]).

IV. Suitability to IUIS

This project aligns closely with the mission of the IUIS by integrating bioengineering, biomechanics, and advanced in vitro modeling to address critical health challenges in liver cancer research. Its interdisciplinary approach—combining microfluidic technology, mechanobiology, and clinical insights—contributes to the development of innovative health technologies, making it well-suited for the IUIS doctoral program.

V. Supervisors and feasibility

This PhD thesis project will be led by **Valérie BELLO (MCU)** and **Wenjin XIAO (CRCN)**, in the team of "Processus dynamiques et multi-échelles de l'organisation spontanée dans la morphogenèse tissulaire", laboratory of Développement Adaptation et Vieillissement (Dev2a), Institut de Biologie Paris-Seine (IBPS), Sorbonne Université. The team specializes in developing application-specific microfluidic models that integrate desired mechanical conditions of fluid flow and substrate viscoelasticity. Currently, the team focuses on improving the pathophysiological relevance of *in vitro* models by leveraging developmental biology processes to guide cells to construct perfusable microvessels. This approach aims to achieve more effective and physiologically relevant vascularization of cultured tissues, ultimately improving *in vitro* platforms for studying the impact of mechanical signals within vessels on tissue homeostasis and disease progression.

Valérie BELLO is an expert in ECM remodeling and biomechanics, with a particular focus on the formation and differentiation of tissue and organs [12]. She recently joined the team in 2025, and is interested in mechanical cues in liver cancer progression, particularly ECM remodelling in tumor angiogenesis. **Wenjin XIAO** is a CNRS researcher recruited in 2024. She has developed a wide expertise in liver biology and model development [13], cancer biology and tumor model development [14], vasculature engineering [15], and vascularized organ-on-a-chip systems. This PhD thesis project will be conducted in collaboration with **Manon ALLAIRE (MCU-PH)**, a hepatologist at Hôpital de la Pitié Salpêtrière, Sorbonne Université. Her research expertise in liver cancer biology [16] will bring critical clinical insights for the project, along with access to patient samples, and validate research findings in clinically relevant models and identify potential therapeutic targets.

The host team is fully equipped with cutting-edge tools and instruments for biofabrication and microfluidics including 3D printers, cutter plotter, UV ozone, pressure generator & controller, microscopy, etc. The host laboratory is fully equipped with cellular and molecular biology that are requested by the project, including a cell culture facility, fluorescent microscope, flow cytometry, 10X genomics, Luminex, etc. Within the IBPS institute, the technology platforms and their experienced engineers will also be beneficial for the project, e.g. imaging, animal facility, etc.

VI. Candidate

The ideal candidate needs to hold a master's degree (or equivalent) in bioengineering, biomedical engineering, cell biology, biophysics, tissue engineering, or a related field. Experience in microfabrication, mammalian cell culture, immunofluorescence, and imaging is highly desirable. Knowledge of mechanobiology, perfusion-based culture systems, and liver models is a plus.

References

[1]. Siegel et al., A Cancer J Clin. 2021, 71: 7. **[2]**. Ong et al., Mol Imaging Biol. 2009, 11: 334-42. **[3]**. Poddar et al., Lab Chip. 2024, 24, 3668. **[4]**. Nashimoto et al., Biomaterials. 2020, 229: 119547. **[5]**. Libby et al. Biomater Biosyst. 2024, 15: 100097. **[6]** Konopka et al., Biosens Bioelectron. 2024, 249: 115986. **[7]**. Lu et al., Lab Chip. 2018, 18: 3379. **[8]**. Nguyen et al. Proc Natl Acad Sci. 2013, 110: 6712–7. **[9]**. Bischel et al. Biomaterials. 2013, 34: 1471–7. **[10]**. Chhabra et al. Proc Natl Acad Sci USA. 2022, 119: e2115867119. **[11]**. Zhang et al. Lab Chip. 2021, 21: 473–488. **[12]**. Buisson et al. Development. 2014, 141: 4569-79. **[13]**. Xiao et al. Integr Biol. 2015, 7: 1412-1422. **[14]**. Källberg et al. Cell Death Dis. 2023, 14: 306. **[15]**. Monroy-Romero et al. ACS Biomater Sci Eng. 2024, 10: 7054-7072. **[16]**. Copil et al. Liver Int. 2024, 44: 931-943.