**Human Biomimetic System RIKEN Hakubi Research Team（2023）**
**Hakubi Teamleader: Masaya Hagiwara (Ph.D)**

**(0) Research fields**

CPR Subcommittee**:** Engineering
**Keywords:** Organoid, Extracellular matrix, Body axis formation, Environmental control

**(1) Long-term goal of laboratory and research background**

When forming mini-organs (organoids) from stem cells, they are typically cultured as aggregates of cells that have been individually dissociated, resulting in the loss of positional information under uniform conditions. Consequently, their morphology often diverges from that of the original organs. This presents challenges in creating models for drug screening, where organ interconnectivity and drug penetration for metabolism and excretion are crucial. Hence, our laboratory is actively researching techniques to provide spatial positional cues to cells by reconstructing the intricate in vivo environment ex vivo. Our goal is to design and regulate organ morphology during organoid production.

**(2) Current research activities (FY2023) and plan**

**(A-1) Platform for Analyzing Organ Interactions**

In recent years, amid increasing scrutiny and reevaluation of animal experimentation worldwide, "Organ-on-a-chip" capable of observing interactions between cells or organs have garnered global attention as a novel alternative to animal testing. In the United States, numerous venture companies have been established, and the FDA is closely monitoring the situation, indicating a trend that is likely to spread worldwide in the near future. However, the current state of " Organ-on-a-chip " still faces many challenges such as the complexity of operation, the specialization required in techniques, and the instability of data, preventing its widespread practical application. Additionally, the development of "organoid formation," which is expected to serve as a new in vitro drug screening system alongside organ chips, is progressing on a separate vector, without proper technological coordination.

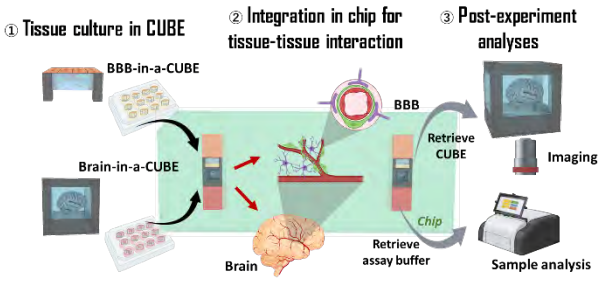


Fig.1: Overview of the platform

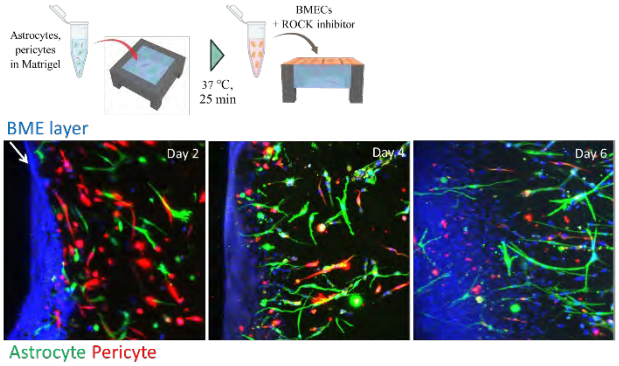


Fig. 2: Overview of the BBB module

To address the aforementioned issues, we have developed a cultivation system called CUBE that enables multidimensional observation of three-dimensional cell tissues from various angles. Leveraging the ease of handling samples with this CUBE system, we have developed an integrated and separable system that combines the cultivation system for cell tissues with the control system of microfluidic chips on demand. By creating a mechanism to introduce CUBEs into the chip cartridge at any desired time, it becomes possible to encapsulate tissue samples from three-dimensional cultures into the chip within seconds. This allows experiments to commence in multi-organ systems without compromising cell function. Additionally, the ability to use cells and tissues cultured externally on the chip beforehand enables the selection and control of high-quality cells (CUBEs), thereby reducing experimental variability significantly.

This year, utilizing this platform, we constructed a blood-brain barrier (BBB) model. To modularize the tissue, we created 5 mm cubic frames called CUBE frames, filled them with Matrigel, and embedded human brain-derived astrocytes and pericytes inside. Subsequently, we seeded brain microvascular endothelial cells differentiated from human induced pluripotent stem cells (iPSCs) onto the surface of the Matrigel. Through co-culturing these three cell types, we successfully reproduced a morphology resembling the blood-brain barrier, where astrocytes and pericytes extend three-dimensionally towards endothelial cells beneath the endothelial cell sheet (Figure 2). Furthermore, confirming its functionality as

a blood-brain barrier, we observed that tight junctions became stronger with increasing culture days, and the expression of major transporter groups of the blood-brain barrier such as PGP and GLUT1 was detected.
To confirm the utility of this platform, we constructed a model depicting the interplay between the blood-brain barrier and brain tumors (Figure 3). Through experiments testing the effects of drugs, we demonstrated its applicability in screening for anticancer agents capable of crossing the blood-brain barrier and entering the brain.

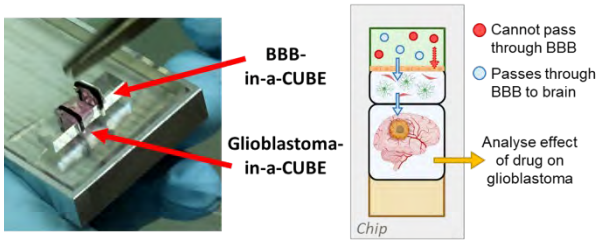

Fig.3：BBB-Gliobrastoma model

Future Plans:
This fiscal year, our main focus was on creating a blood-brain barrier (BBB) module primarily for analyzing pharmacokinetics. However, the BBB itself is directly or indirectly involved in various diseases, and there are still many aspects of its interaction with the brain that remain unclear. Moving forward, we plan to position neuronal organoids downstream of the BBB and further advance the construction of disease models.

The CUBE-type culture system we've developed enables manipulations like grasping organoids with forceps along with their extracellular matrix during cultivation and transferring them to another container. Leveraging this ease of handling, we utilize the CUBE as a carrier for organoids. At the mature stage of organoids within the CUBE, we encapsulate them, along with the CUBE, into PDMS-made fluidic chips. By positioning the organoid-containing CUBE in the center of chamber and introducing culture medium containing different growth factors into the chambers of both sides of the CUBE, we establish a gradient of factors within the CUBE, termed the "Gradient-in-CUBE system." Using this system, we positioned clusters of iPSCs cultured for several days at the center of the CUBE, and after five days of culture with neural ectoderm and mesoderm differentiation factors added to their respective chambers within the fluidic chip, imaging post-sectioning revealed the localization of differentiation markers Brachyury and SOX2 for neural ectoderm and mesoderm, respectively, within a single cell cluster (Figure 4).

***Future plan***) Using this platform, we aim to control the morphology of organoids and expand them into drug screening models. Additionally, by combining multiple CUBEs, we are advancing the development of in vitro models that enable the analysis of inter-tissue interactions.

**(3) Members**

**(Hakubi Team Leader)**
Masaya Hagiwara
**(SPDR)**
Raja Vadivelu
**(Postdoctoral Resercher)**
Isabel Siew Yin Koh
**(Postdoctoral Researcher)**
Yen Ngo
**(Assistant)**
Satoko Kinoshita

**(4) Representative research achievements**

1. Koh I., *Hagiwara M., Modular tissue-in-a-CUBE platform to model blood-brain barrier (BBB) and brain interaction, Communications Biology, 7, 177, 2024.

Laboratory Homepage
https://hbms.riken.jp/