Human Biomimetic System RIKEN Hakubi Research Team（2022）
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 (FY2022) and plan

(A-1) Localization of Extracellular Matrix for symmetry breaking of organoids

The extracellular matrix (ECM), composed of non-cellular materials like collagen, encircles cells and significantly influences organ growth through mechanical and biochemical signaling. ECM's structure and composition vary around organs, offering positional cues to cells as they organize into tissues. However, prevailing cell culture techniques often lack spatial precision due to the uniform distribution of ECM around cells. This year, we introduced MultiCUBE, a flexible hydrogel processing method, to recreate ECM's spatial complexity ex vivo, enabling the generation of multiple gels (Fig. 1).

We created a platform where multiple spaces (units), each surrounded by an L-shaped frame, hold gels using only surface tension, allowing for spatial arrangement of multiple gels. While the gels in adjacent units are in contact, their high viscosity prevents immediate mixing. After arranging the gels, they are cross-linked and solidified. This creates a physical boundary-free environment between the gels, enabling cell movement, while also generating different compositions within the gels based on their positions, providing spatial information to cells. Consequently, not only can gels with different properties be spatially arranged, but by pre-mixing specific growth factors or cells into the gels, cells can be given spatial localization of multiple elements.

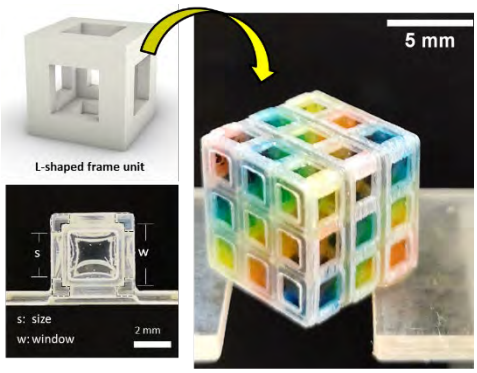

Fig.1: Overview of MultiCUBE

With this platform, we positioned gels containing a mixture of heparin-bound EGF-like growth factors next to gels without any additives. Cultivating bronchial epithelial cells in a single row resulted in the exclusive formation of numerous branches within the units containing the growth factors, while the others aggregated to form a rod-like shape, resulting in an asymmetric tissue structure (Fig. 2). Thus, by artificially creating spatial localization of growth factors within the cellular microenvironment, we were able to manipulate the process of inducing isolated individual cells into multicellular tissues spatially, showcasing the capacity to steer them toward more intricate morphologies.

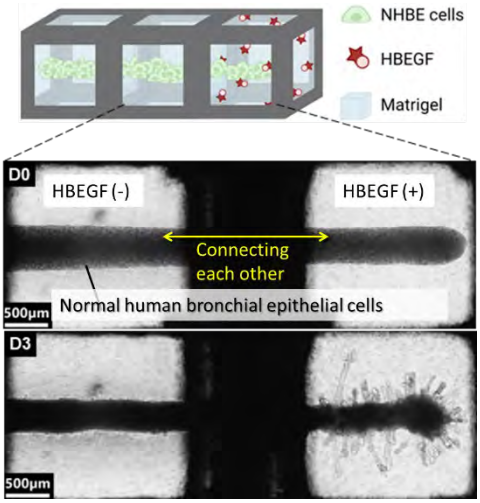

Fig.2: Asymmetric pattern formation by localizing growth factor

(A-2) Morphogen gradient control

During organ development, cells interpret concentration gradients of specific factors in their surroundings as positional cues, establishing body axes like dorsal-ventral, anterior-posterior, and left-right. Consequently, generating concentration gradients of factors in ex vivo culture experiments is vital for driving organoids to develop into more complex structures. In this study, we devised a straightforward

approach to induce the formation of body axes by exposing cells to concentration gradients of factors. This method allows for the creation of organoids that retain gradient information and can be analyzed effectively (Figure 3).

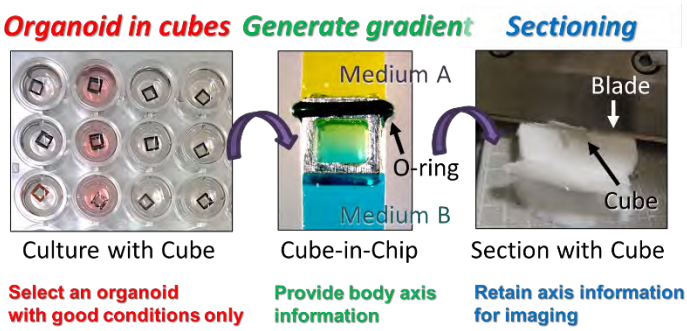

Fig. 3: Workflow of Gradient-in-CUBE system

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).

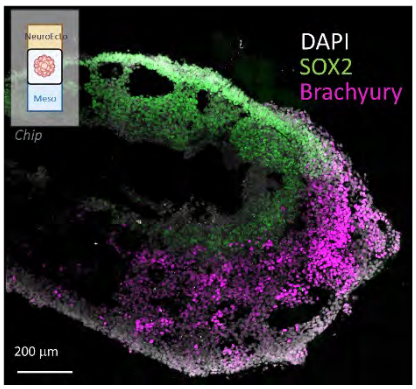

Fig. 4: Localization of differentiation markers by morphogen gradient

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

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(4) Representative research achievements

1. Koh I., *Hagiwara M., Gradient to sectioning CUBE workflow for the generation and imaging of organoids with localized differentiation, Communications Biology, 6, 299, 2023.
2. Suthiwanich K., *Hagiwara M., Localization of multiple hydrogels with MultiCUBE platform spatially guides 3D tissue morphogenesis in vitro, Advanced Materials Technologies, 8, 4, 2023.

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