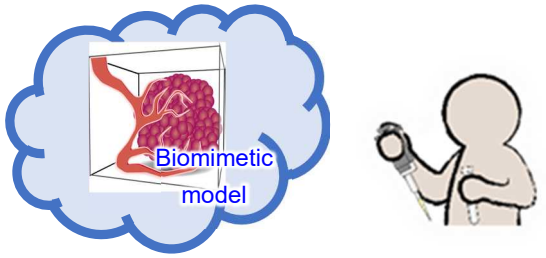
R&D Item

6. Evaluating remote control of intracellular CA in a simulated *in vivo* environment

Progress until FY2024

1. Outline of the project



To analyze whether the intracellular Cybernetic Avatar (hereinafter referred to as "intracellular CA") functions as designed, R&D Item 6 will focus on developing a three-dimensional (3D) biomimetic model and integrating a measurement and evaluation system for assessing the behavior of CA-loaded cells. Through this integration, we aim to establish an evaluation platform for CA-loaded cells.

The evaluation technology developed in this R&D Item 6 will enable quantitative assessment of CA-loaded cells as an alternative to *in vivo* evaluation, which inherently involves black-box elements. Furthermore, by linking this technology with the culture environment evaluation in R&D Item 4 and the *in vivo* evaluation in R&D Item 5, it will contribute not only to reducing the use of laboratory animals but also to shortening the development time of intracellular CA technologies.

Specifically, in R&D Item (6-1), we will develop 3D biomimetic models capable of evaluating the collective behavior of CA-loaded cells. In R&D Item (6-2), we will develop environment control technologies that enable time-lapse observation of CA-loaded cells and integrate them with the measurement and evaluation system to construct the evaluation platform.

(6-1)
Development of 3D in-vivo Simulated Model for Evaluating Teleoperation of Intracellular CAs
Takeshi Hayakawa
Chuo University

(6-2)
Development of 3D in-vivo Simulated Platforms for Evaluating Teleoperation of Intracellular CAs
Shinya Sakuma
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2. Outcome so far

In R&D Item 6, we aim to develop a 3D biomimetic model and platform to evaluate the remote-controlled Start, Conditional branching, and Stop of the process in which immune cells equipped with intracellular CAs for inspection or remove recognize and selectively inspect or remove target cells. To date, we have conducted the following studies in each Item.

(6-1) We have started the development of a platform for evaluating the interactions and remote controllability of CA-loaded cells (Figure 1). We began selecting materials and processing conditions for the 3D biomimetic model and created prototype models. These models were embedded in microfluidic devices with high environmental controllability, and we successfully cultured cells within these devices. Furthermore, we evaluated the viability of the cells cultured in the devices and confirmed that they could be maintained with high survival rates.

(6-2) We designed and prototyped the fluid control system and the injection system for CA-loaed cells, which are part of the evaluation platform for the interaction and remote control of CA-loaded cells without the use of experimental animals. We verified their basic functions. Additionally, we implemented fluid and environmental control within the microfluidic devices created in R&D Item 6-1, and prototyped systems capable of simulating the in vivo environment.

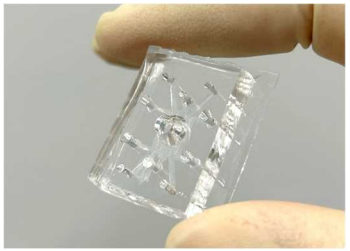

Figure 1 Prototyped microfluidic device

3. Future plans

By seeding and culturing target cells within a 3D biomimetic model, and introducing inspection or remove CA-loaded cells, we will observe and evaluate the remote controllability of the Start, Conditional branching, and Stop of the inspection or removal process.

Specifically, we will complete the development of a 3D biomimetic model that mimics the target tissue of this project (currently under prototyping), and use an evaluation platform integrated with fluid and environmental control systems to assess the functionality of CA-loaded cells. Furthermore, we will deploy these technologies both within and outside the project to improve usability, stability, and other aspects, thereby accelerating the overall progress of the project.

In addition to evaluating intracellular CAs and CA-loaded cells, we will also develop devices that visualize the activity of intracellular CAs and CA-loaded cells, and actively disseminate information through outreach activities and other means.