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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）モジュール化オルガノイドの血管形成制御による多臓器相互作用モデルの構築

研究課題名（英文）Construction of a multi-organ interaction model via the controlled vascularisation of modular organoids

研究代表者

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研究成果の概要（和文）：オルガノイドおよびOrgan-on-a-Chip（OoC）技術は現在、体外でヒトの組織を再現するために進歩していますが、両方の技術を組み合わせるのは困難である。 本研究では、複雑なパターンを作成するための溶けるモールド又は単純な播種ポケットを作成するための樹脂ベースのモールドを用いて、CUBE 培養装置内の細胞の播種位置とパターンを制御できる方法が開発された。CUBEのモジュール性により、細胞をOoCと簡単に統合して、人体と同様の培養条件を模倣できます。体内の異なる組織への細胞分化を誘導するシグナル伝達勾配と同様、成長因子の勾配を用いてiPS細胞を培養することによって実証された。

研究成果の学術的意義や社会的意義
The methods developed in this research can help bridge the gap between organoid and OoC researchers, leading to the generation of more sophisticated organoids that can mimic human organs, which would be useful in studying human disease mechanisms and drug treatment.

研究成果の概要（英文）：Organoid and Organ-on-a-Chip (OoC) technologies are currently being advanced to replicate the human body in the laboratory, but it is still difficult to combine both technologies together. In this research, two methods to control the seeding position and pattern of cells in a CUBE culture device were developed: (1) 3D bioprinting of dissolvable carbohydrate glass mould to create complex patterns in hydrogel, and (2) 3D printed resin-based mould to create simple seeding pocket in hydrogel. Due to the modularity of the CUBE device, the cells can then easily be integrated with OoC devices to simulate culture conditions similar to that in the human body. This was demonstrated by culturing cells with a gradient of growth factors from opposing directions, similar to the signalling gradients in vivo that guide cell differentiation into different tissues or organs in the body.

研究分野：Tissue Engineering

キーワード：Organ-on-a-Chip　Organoid　3D culture　Organ-organ interaction

１．研究開始当初の背景

Research efforts to replicate the human body *in vitro* have led to major advances in the culture of organoids that mimic *in vivo* organs, as well as Organ-in-a-Chip (OoC) technologies that simulate physiological environmental conditions. The combination of both these technologies together has the potential to greatly enhance the representation of the human body in the laboratory. Yet, several problems exist that make this task difficult. The maturation potential of organoids is greatly hampered by the lack of nutrient supply able to reach the inner regions of the organoid, leading to undifferentiated or necrotic cores. Furthermore, it is difficult to link multiple organoids together to enable the study of organ-organ interactions. On the other hand, several OoCs have been developed using principles of microfluidics to imitate the flow of blood in delivering nutrients to cells and connecting different organs in the body. However, organoids are very delicate samples that are not easy to handle by pipetting without damaging their structure, making it difficult to incorporate them into OoCs. Additionally, current microfluidic devices are complicated to set up and not easily adopted by non-engineering researchers as they often require lengthy manufacturing processes and pump systems.

We had previously developed a CUBE culture device consisting of a polycarbonate frame with agarose gel walls that contained an inner hydrogel compartment to support the culture of cells or tissue samples in 3D (Hagiwara *et al.*, 2016). With the organoid suspended in the inner extracellular matrix (ECM) gel in the CUBE, the rigidity of the frame and agarose gels enable the sample to be picked up using a pair of tweezers but still allows nutrients from culture media to diffuse into the inner sample. The improved handling ability of organoids by using the CUBE enables us to pick up the organoid and incorporate it into an OoC device with ease, as well as apply precise engineering techniques to the sample without damaging the sample.

２．研究の目的

The aim of this research was to integrate the use of the CUBE culture device with simple OoCs that require minimal processing and setup times to replicate physiological conditions for organoids *in vitro*. To achieve this, the objectives were to develop methods to control the seeding position of cells or organoids in the CUBE device, vascularize organoids, and incorporate the organoid-containing CUBE in an OoC.

３．研究の方法

The rigidity and consistent shape of the CUBE frame facilitates the design of tools to enable precise seeding of cells in the CUBE. Two methods were developed to control the cell seeding position in the CUBE:

(1) 3D bioprinting of dissolvable carbohydrate glass mould

Carbohydrate glass is a bio-printable material that can be easily dissolved in water after it has been printed (Miller *et al.*, 2012). Utilizing this material, moulds of complex shapes were printed and embedded in the hydrogel contained in the CUBE. Once the gel has been cured, the mould was dissolved by soaking the CUBE in water, thus leaving a pocket in the shape of the mould in the hydrogel, in which endothelial cells were then seeded to create vascularized constructs.

(2) 3D-printed resin mould cap

Carbohydrate glass moulds are great for creating complex patterns in hydrogels, but the bioprinting process requires many adjustments of various parameters such as extrusion pressure, printing speed, nozzle sizes, and so on. For simple designs, it is more suitable to use resin-based materials that are more durable and easier to print. In this case, a simple pillar-shaped mould was 3D-printed and placed in uncured hydrogel in the CUBE as before. After the gel has cured, the mould was gently removed from the gel to leave a pocket into which cell spheroids were seeded.

With both methods, the position of the mould can be precisely controlled by designing the mould cap to fit on the frame of the CUBE, with the structure of the mould pattern in the desired position when placed on the CUBE (Fig. 1A, Koh *et al.*, 2023).

The CUBE device also allows the easy incorporation of organoids into OoC devices, as the fragile organoid samples are encased in the CUBE and can be picked up using tweezers

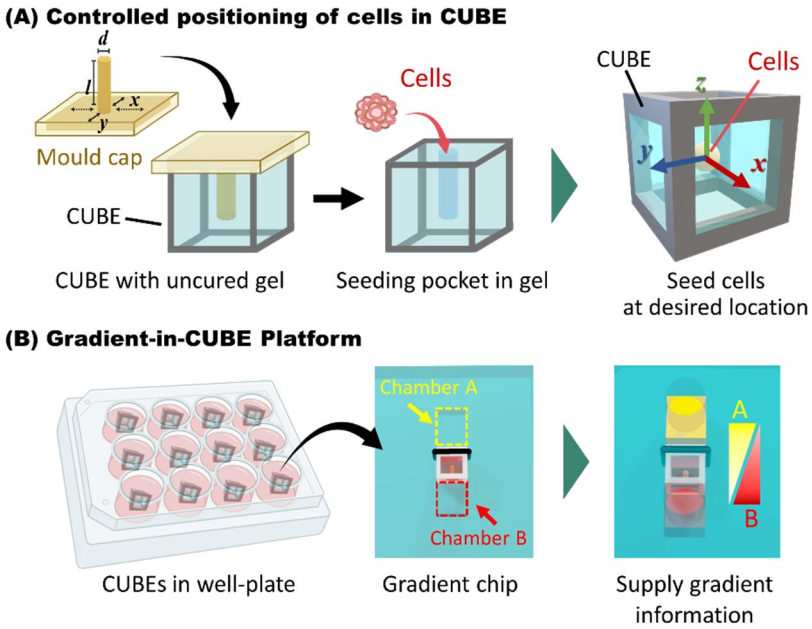

Figure 1. (A) By using a 3D-printed mould, a seeding pocket can be created in hydrogel in the CUBE to control the seeding position of cells in the desired position in the CUBE. (B) CUBE containing cell sample can be cultured regularly in a well-plate, then transferred to an OoC device to culture the cells under gradient conditions.

without damaging the sample. In this project, the integration of CUBE and OoC device was demonstrated by a gradient-forming chip device with compartments to contain the CUBE in the middle and two medium chambers on opposite sides of the CUBE (Fig. 1B, Koh *et al.*, 2023).

4．研究成果

Carbohydrate glass can be used to print 3D moulds with complex designs and used to create seeding pockets of the desired pattern in hydrogels. In this study, a double helix-shaped pattern was made in collagen hydrogel in the CUBE (Fig. 2A, Takano *et al.*, 2022). Then, endothelial cells were seeded in the helical channels via the inlets to mimic vascular channels (Fig. 2B, Takano *et al.*, 2022). Another advantage of the CUBE device is that the CUBE can be rotated to image large samples from multiple directions and the images overlaid with each other gives an image of the whole sample.

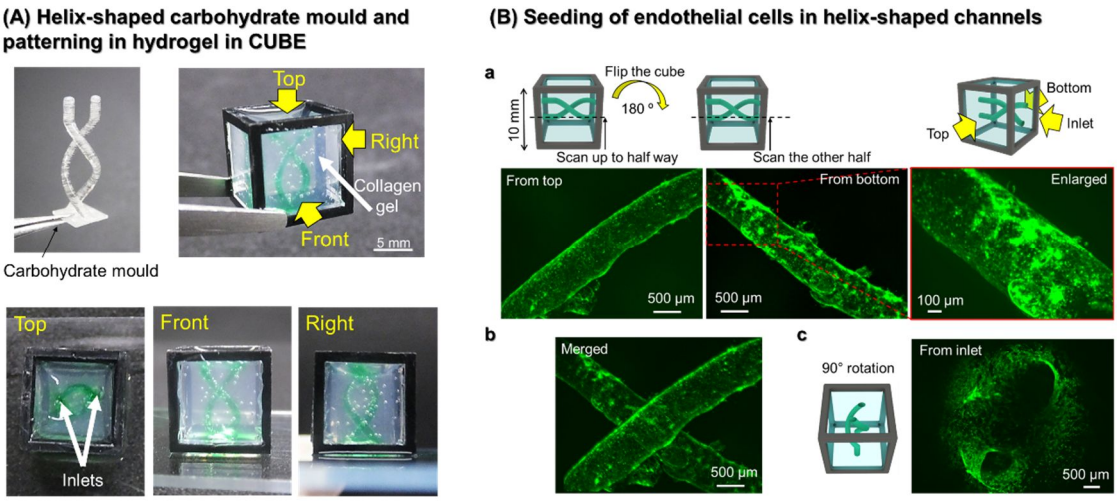

Figure 2. (A) Helix-shaped mould printed using carbohydrate glass can be used to create complex seeding patterns in the hydrogel in the CUBE. (B) Seeding of endothelial cells in the pattern created in the hydrogel generates vascular-like structures.

For simple positioning of cells in the CUBE, resin-based mould cap can be used to create a seeding pocket in the hydrogel to consistently place cells or spheroids in the desired position in the CUBE (Fig. 3, Koh *et al.*, 2023). After the spheroid has been seeded in the

CUBE, the sample can be integrated into a chip made of PDMS with two separate medium chambers on either side of the CUBE to generate a gradient of opposing media across the spheroid in the CUBE (Fig. 4A, Koh *et al.*, 2023). The application of this gradient culture was demonstrated by culturing a single iPSC spheroid with two types of differentiation media to generate a spheroid with localized differentiated regions (Fig. 4B, Koh *et al.*, 2023). Due to the modularity of the CUBE device, cells can be cultured in a regular well-plate before being transferred to the gradient chip at the desired timing, allowing control over differentiation periods.

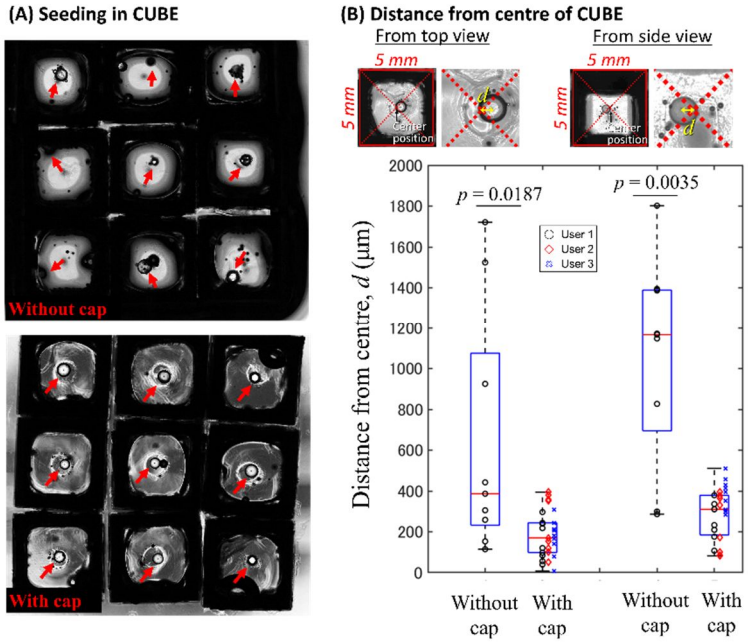

Figure 4. Seeding of spheroids in the CUBE with the use of mould to guide the position of spheroids during seeding resulted in more consistent and accurate positioning of spheroid in the desired position in the CUBE compared to without using the mould.

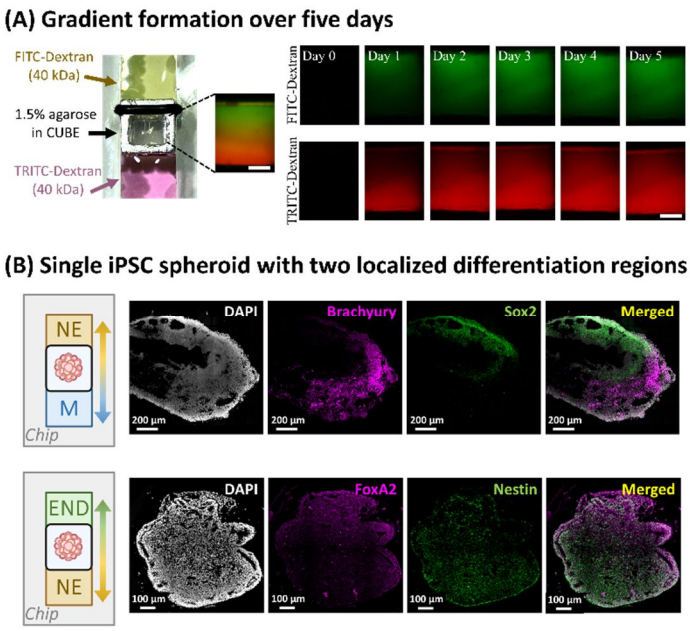

Figure 3. (A) Integration of CUBE with gradient chip enables the generation of a gradient of two different types of media. (B) iPSC spheroid cultured with two types of differentiation media expressed two localized differentiation regions.

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〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　2件）

１．著者名 Takano Atsushi、Koh Isabel、Hagiwara Masaya	４．巻 13
２．論文標題 3D Culture Platform for Enabling Large-Scale Imaging and Control of Cell Distribution into Complex Shapes by Combining 3D Printing with a Cube Device	５．発行年 2022年
３．雑誌名 Micromachines	６．最初と最後の頁 156
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１．著者名 Koh Isabel、Hagiwara Masaya	４．巻 6
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オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

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４．発表年 2022年

〔図書〕　計0件

〔出願〕　計1件

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特許、特願022-140997	2022年	国内

〔取得〕　計0件

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関