2024 年度 SP+ Fund 報告書（General）

Project Report: SP+ Fund 2024 (General Program)

プロジェクトの基本情報／Key Project Information	
課題名（英語） Project name (in English)	Decellularized liver matrix-based self-healing hydrogel containing hepatocyte spheroids for the rescue of iPSC-derived non-alcoholic fatty liver cells
期間／Period	From 2024/07/01 Until 2025/03/15
主な研究分野 Main research fields	Liver tissue engineering
活動内容 （該当するものに全て✓してください。） Activities to be funded (check ✓ all applicable items)	☑ 研究ワークショップ、会議、ラウンドテーブル、シンポジウム等の実施／Research workshops, conferences, roundtables, symposiums, etc. ☑ 共同研究や研究打合せにかかる渡航・招へい Travel/invitations for collaborative research or research meetings ☐ その他 （具体的に）／Other (please specify) ( )
区分／Type of collaboration	☑ Bilateral ※本学と SP 校との 2 機関で実施するプロジェクト (Project conducted by Kyoto University and one SP institution) ☐ Multilateral ※本学と SP 校に加え、さらに 1 機関以上 (Project conducted by Kyoto University, an SP institution, and one or more additional institutions)
実施場所／Location of implementation	☑ 京都大学／Kyoto University ☐ その他／Other location ( )

申請者（京都大学）／Applicant (Kyoto University)	
姓／Family name	Kenji
名／Given name	Osafune
職名／Position	Professor
所属部局 Faculty/dept. of affiliation	Center for iPS Cell Research and Application (CiRA)

SP 校のプロジェクト代表者／Representative from SP institution	
姓／Family name	Hou
名／Given name	Yung-Te
職名／Position	Professor
所属大学／Institution	☐ ボルドー大学／University of Bordeaux ☐ ウィーン大学／University of Vienna

SP 校のプロジェクト代表者／Representative from SP institution	
	☐ チューリヒ大学／University of Zurich ☐ ハンブルク大学／University of Hamburg ☑ 国立台湾大学／National Taiwan University
所属部局 Faculty/dept. of affiliation	Department of Biomechatronics Engineering

その他のプロジェクト代表者（Multi の場合）／Representative from other collaborating institution (in the case of multilateral projects) ※	
姓／Family name	
名／Given name	
職名／Position	
所属大学／ Institution	☐ ボルドー大学／University of Bordeaux ☐ ウィーン大学／University of Vienna ☐ チューリヒ大学／University of Zurich ☐ ハンブルク大学／University of Hamburg ☐ 国立台湾大学／National Taiwan University ☐ その他／Other （機関名／name of institution: ）
所属部局 Faculty/dept. of affiliation	

※4機関以上によるプロジェクトの場合は、必要に応じて欄を追加願います。
If the project involves four or more institutions, please insert additional fields as required.

プロジェクトの実施内容／Summary of the project

公開されている関連リンクや、フライヤー、プログラム、報告書、広報記事等の提出をもってして代えることも可能です。 This could be substituted by submitting publicly available relevant links, flyers, programs, reports, publicity articles, etc.

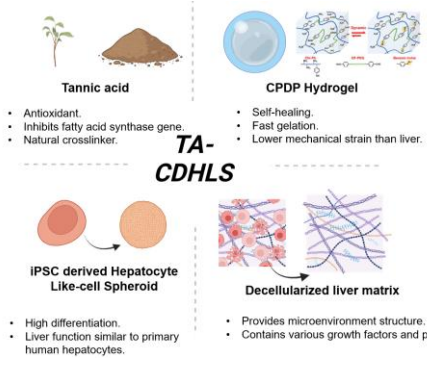
Part I (ECR student: Xin-Yu Chou) (Main part of this project)

Currently, no FDA-approved drugs are available for MASLD, and patients can only manage their condition through lifestyle modifications, such as exercise and dietary changes. Consequently, MASLD remains highly prone to recurrence. Therefore, this study aims to develop a novel treatment for MASLD by integrating iPSC-derived Hepatocyte-Like Cell (HLC) spheroids with Tannic Acid (TA) into a decellularized liver matrix (DLM)-based CPDP self-healing hydrogel, forming TA-CDHLS (TA + CPDP + DLM + HLC spheroids), as illustrated in **Fig. 1A**.

During the first month of the study, efforts were directed toward learning iPSC culture techniques and differentiating them into HLC spheroids using a three-stage differentiation protocol. The hepatic functions of cells cultured in 2D and 3D systems were then compared by assessing urea synthesis, albumin synthesis, and DNA expression. In the second month, mass-cultured HLC spheroids were incorporated into CPDP hydrogel, which was subsequently applied to treat an MASLD model. The therapeutic effects of TA-CDHLS were evaluated based on LDH activity, urea synthesis, albumin synthesis, and DNA expression. The overall two-month experimental plan is depicted in **Fig. 1B**.

The key findings of this study include: (1) Hepatic functions of 3D-cultured HLC spheroids were superior to those of 2D-cultured cells, highlighting the therapeutic potential of HLC spheroids incorporated into CPDP hydrogel. (2) In groups supplemented with TA (0.1 wt%, 1.0 wt%, 10 wt%), spheroids within the hydrogel maintained an intact and compact structure. This effect is attributed to TA's role as a natural crosslinker, which enhances the mechanical strength of the CPDP hydrogel and preserves spheroid integrity. (3) TA-CDHLS demonstrated higher DNA content, urea synthesis, and albumin synthesis, along with lower LDH activity compared to the Control group (CDS). Notably, the 10 wt% TA-CDHLS exhibited the most significant therapeutic effects. These results confirm the therapeutic potential of TA-CDHLS as a promising approach for MASLD treatment.

(A)



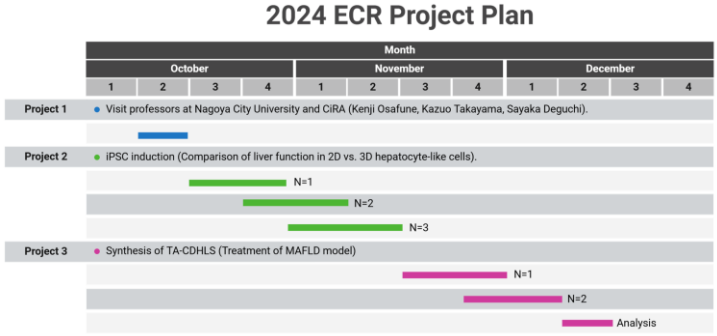


Fig. 1. Summary and timeline of the SP + Fund 2024 (General program). (A) The experimental flowchart; (B) Experimental timeline.

Part II (ECR student: Yu-Hua Lin) (Supporting part of this project)

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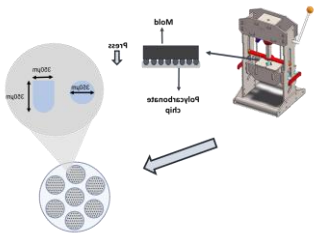
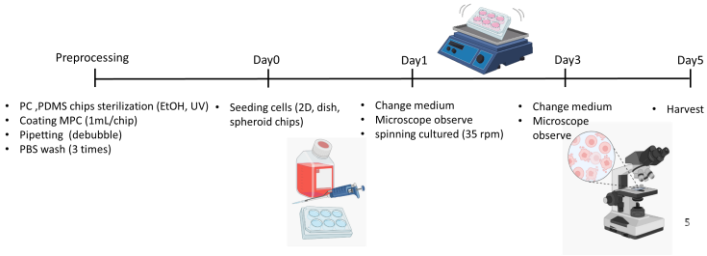
During the exchange period, this supporting part aimed to produce a large quantity of HLC spheroids to supply the main experiments conducted in Part I, utilizing the device and method previously developed at NTU (**Fig. 2A**). Different materials and spheroid chip designs, such as polycarbonate (PC) and polydimethylsiloxane (PDMS), were tested, along with variations in microwell size and shape. To enhance spheroid chip production efficiency, a rigid imprinting technique was employed, allowing for the rapid fabrication of large quantities of uniform microwells in a single step (within 10 seconds). Additionally, to facilitate cell aggregation, microwells were designed in a tubular shape. The experimental flowchart for spheroid cultures using the spheroid chip is illustrated in **Fig. 2B**.

Next, we focused on optimizing the process for culturing HLC spheroids at KU, which required approximately two weeks and involved three distinct stages. The quality of HLC spheroids was assessed based on DNA content, urea synthesis, and albumin synthesis (**Fig. 2C**). Furthermore, to enhance the efficiency of HLC spheroid production using our spheroid chip, the cultures were subjected to continuous rotation at a speed of approximately 30–35 rpm (adjusted according to cell conditions) for four days. Under these conditions, cells successfully aggregated into uniformly shaped HLC spheroids. By maintaining a specific rotational speed on an orbital shaker, the spheroid chip was capable of producing 1,000 HLC spheroids per batch with a high recovery rate (~90%). The progression of HLC spheroid formation over different culture durations within the spheroid chip is presented in **Fig. 2D**.

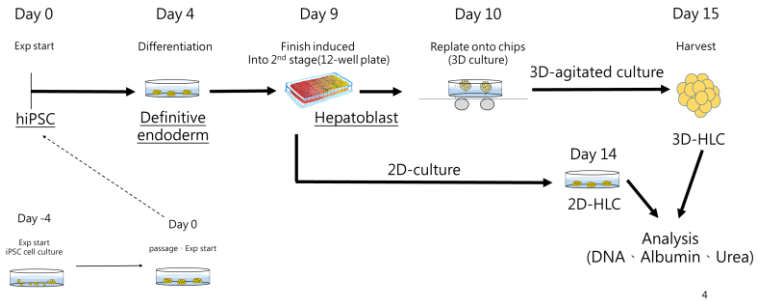
(A)



(B)



(C)



(D)

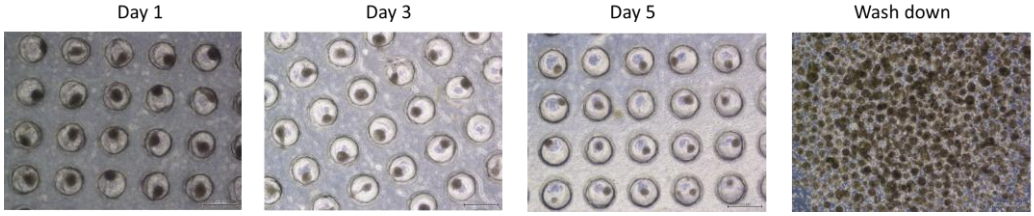


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Fig. 2. (A) Design of spheroid chips; (B) The experimental flowchart of spheroid cultures in spheroid chip; (C) The experimental flowchart of HLC spheroid cultures in spheroid chip; (D) The progression of HLC spheroid formation over different culture durations within the spheroid chip.

今後の展望／Prospects for future research collaboration

The KU team and NTU team have established a strong collaborative relationship through multiple research exchanges. In 2023, two master students participated in an exchange program at CiRA from July to August (Fig. 3A), followed by the 1st NTU/CiRA Joint Symposium held at NTU in December (Fig. 3B). The collaboration continued in 2024 with another exchange program from October to December, where another two master students visited CiRA once again (Fig. 3C). This was accompanied by the 2nd NTU/CiRA Joint Symposium in late December (Fig. 3D). These academic exchanges and collaborative activities (Fig. 3E) have significantly strengthened the research relationship between the two teams, fostering technological innovation and advancing academic progress.

To further enhance this collaboration, we plan to invite Prof. Cheng's group (tzongjih@ntu.edu.tw) to provide expertise in designing cell culture systems using microwell PC chips, which could facilitate large-scale spheroid production. Additionally, we seek support from Prof. Chen's group (rlcchen@ntu.edu.tw) for structural and material analysis using biosensing technologies. Furthermore, we aim to engage Prof. Hsieh's group (hsiehpc@ntu.edu.tw) to explore additional applications of iPSCs in food science and food safety, as differentiated iPSC-derived ingredients could contribute to food diversification (Fig. 3F).

Looking ahead to 2025, we aim to strengthen our collaborative research between the KU and NTU teams and organize the 3rd NTU/CiRA Joint Symposium in Taiwan. By integrating KU's expertise in iPSC culture techniques with NTU's innovative liver tissue engineering technologies—including liver patches, nanomedicine, self-healing hydrogels, microfluidics, PC chip spheroid culture, biosensing technologies, and food safety applications—we aspire to jointly address the current challenges in liver disease treatment. This strategic collaboration is expected to accelerate technological advancements and contribute significantly to the field.

Fig. 3. Collaborative activities between KU team and NTU team in 2024 and 2025. (A) 2024 ECR Project; (B) The 1st NTU/CiRA Joint Symposium in 2024; (C) 2025 ECR Project; (D) The 2nd NTU/CiRA Joint Symposium in 2025; (E) Academic collaboration among experts from various fields (from left: Prof. Hsieh, Prof. Hou, Prof. Osafune, Prof. Chen, and Prof. Cheng); (F) Research overviews of experts from different fields.