様　式　C－１９、F－１９－１、Z－１９　（共通）

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研究課題名（和文）疾患スクリーニングのための生理的肝臓モデル培養系の構築

研究課題名（英文）Establishment of a physiologically-relevant liver model for disease screening

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：倫理的な問題や種の特異性から、薬物スクリーニングのための肝臓のin vitroモデルの開発が進められています。肝臓を試験管内で再現することは、その複雑さゆえに特に困難です。ここでは、炎症制御に関与するものの、試験管内で培養すると自然に活性化してしまう肝星細胞の発生に焦点を当てました。肝星細胞の微小環境は、硬さと組成の観点から、異なるECMを介して最適化されました。静止状態を維持できる条件を見出し、炎症性サイトカインを介した活性化の制御も確認した。また、これらの細胞を分化させるためのプロトコルも、再現性の観点から最適化された。共培養も実施し、現在さらに研究を進めている。

研究成果の学術的意義や社会的意義
Physiologically-relevant models are highly sought for in notably drug screening applications but spontaneous activation of HSCs in vitro forces models towards pathological situation. In this work, we have focused on optimizing the culture of HSCs to regulate inflammatory reactions within the model.

研究成果の概要（英文）：Ethical issues and species specificity have motivated the development of in vitro models of the liver for drug screening applications. Modeling of the liver is especially challenging due to its complexity, being composed of hierarchically organized hepatocytes, LSECs, and HSCs. Here, the focus was set on the development of the latter which are known to be involved in inflammatory regulation but spontaneously activate when cultured in vitro, causing issues in the modeling of physiology. To solve the issue, optimization of the microenvironment of HSCs in term of stiffness and composition was done via different ECM. By doing so, optimized conditions in which quiescence could be preserved were found and controlled activation, via inflammatory cytokines was also confirmed. Protocols for the differentiation of those cells were also optimized in terms of reproducibility. Coculture of the previously cited cell types was also performed in microfluidic biochips and are currently further studied.

研究分野： Bioengineering

キーワード： hiPSCs　HSCs　Liver　Disease　LSECs　Hepatocytes　Inflammation　Immune

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様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

The raise in the occurrence of liver disorders has forced to consider the liver not only as a metabolic organ but also as an immunologic organ. In that regard, the involvement of cells such as Hepatic Stellate Cells (HSCs) and Kupffer Cells (KCs) in the inflammation process and in immune reaction has become a central topic. Those cells can be found in close contact with other cells of the liver microvasculature (Figure 1) and are now widely known to be involved in both fibrosis and steatosis. In that aspect, an increasing number of models have been including those cells, notably in the form of spheroids (Baze et al., Tissue Eng. Part C-ME., 2018; Suurmond et al., Adv. Healthcare Mater., 2019; Li et al., J. Pharm. Sci., 2020). Yet, those models critically fail to reproduce the hierarchical structure of the liver microvasculature which is a central point in immune reactions. Another common approach to solve this issue is based on sandwich collagen cultures and studies have included major cell types of the liver and have led to an improved functionality (Bale et al., Sci. Rep., 2016). However, the lack of direct oxygenation does not allow to reproduce the thick multi-layer structure of the liver while maintaining the cell phenotype. Finally, most of the models proposed often lack in cellular complexity or do not use human cells.

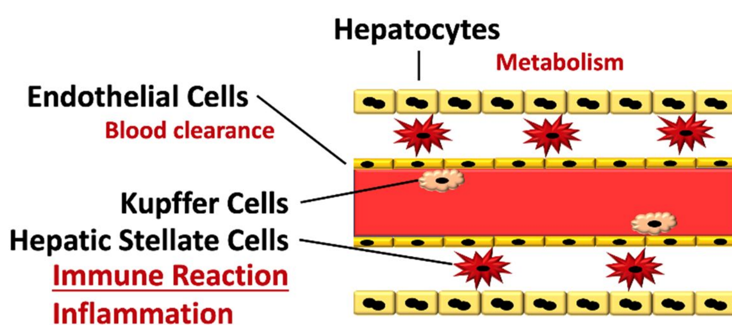

Figure 1: The hierarchical structure of the liver

２．研究の目的

The objective of this study was to establish a coculture model with relevant human cell models which mimics the in-vivo situation. We aimed to apply it to disease modeling such as steatosis, to confirm its physiological relevancy, and to prove the ability to perform the related drug screening.

３．研究の方法

In the first stage of the project, HSCs were derived from hiPSCs following previously detailed protocols (Koui et al., 2017). Differentiation of those cells being based on the culture of spheroids; a certain variability was found in relation to the size and density of spheroids which were formed randomly. In that regard, the formation of the said spheroids in microwells was studied to stabilize the protocol before further investigations. Additionally, the culture

environment of hiPSCs-derived HSCs was optimized both in terms of composition and stiffness. For that purpose, both collagen type-I and Matrigel (mixture which contains notable collagen type-IV) were investigated. The possibility to activate those cells via external stimulations was also tested via cytokines such as TGFB to confirm whether disease modeling could be performed in future experiments. As hepatocytes and LSECs were previously established and their cultures were already optimized in coculture, no further experiments were needed in regard to those cell types. Finally, coculture between the three cell types was performed within a microfluidic biochip and characterization of each cell type was performed separately.

４．研究成果

Optimization of the HSCs differentiation

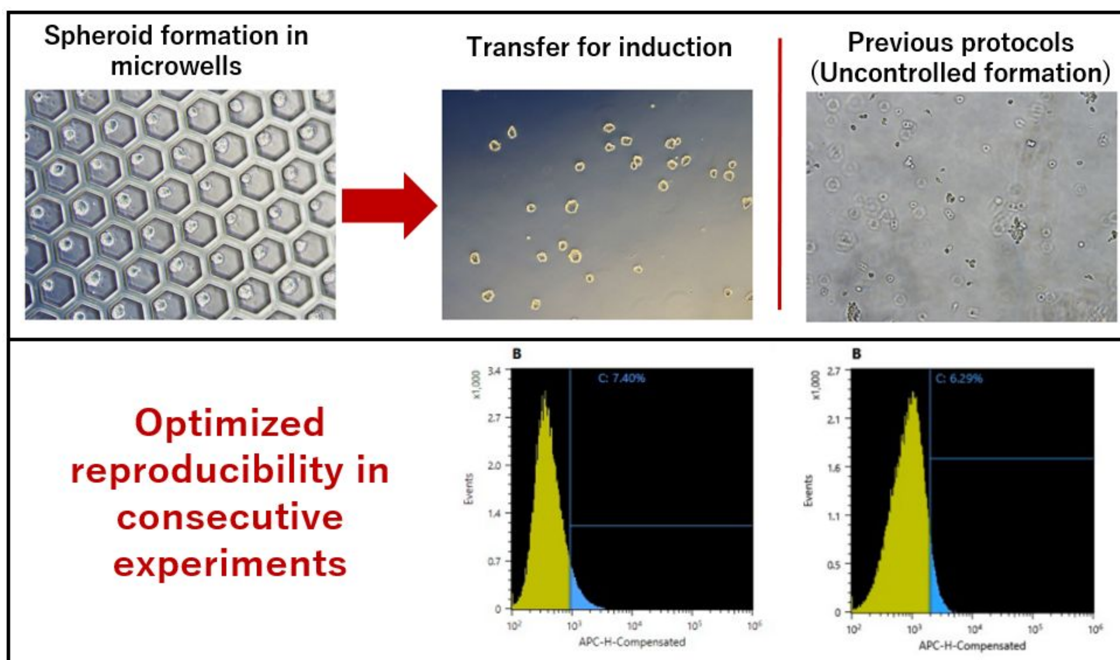

Figure 2: Optimization of the reproducibility of the differentiation protocol

Operator dependency was found to be a serious issue in the previously established differentiation protocols (Koui et al., 2017) and motivated optimization toward more reliable methods. In that regard, as the differentiation is performed in suspension culture of spheroids, the size of spheroids was found to be critical. Spheroids were previously randomly formed in static culture which led to variation between batches of experiments. To solve the issue, spheroids were pre-formed in PDMS honeycomb microwells as previously described (Shinohara et al., 2014) before transfer for induction (Fig. 2). This protocol led to the formation

of rather homogeneous spheroids in which hiPSCs-derived HSCs were found to represent 6-7% of the cell total in consecutive independent experiments. The yield was found to be sometimes higher in the previous protocol (Up to 35%) but also sometimes as low a 1-2%. Further optimizations of the protocols are currently being investigated to improve the yield of the differentiation.

Optimization of the HSCs culture

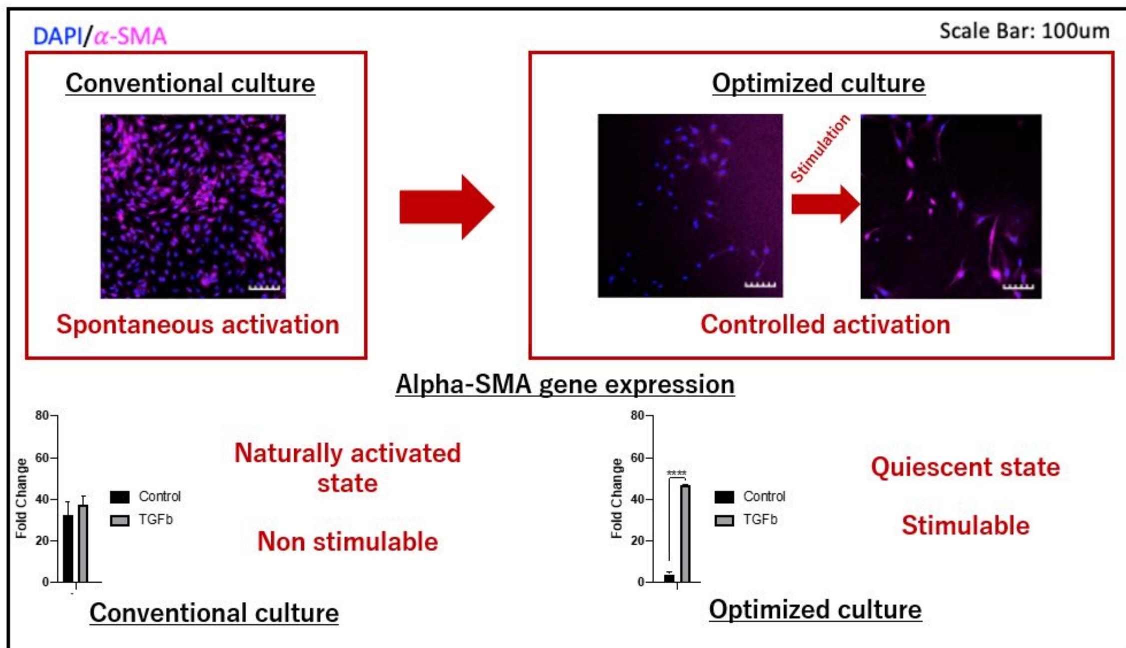

Figure 3: Optimization of the control over HSCs activation *in vitro*

As previously discussed, one of the major issues in the addition of HSCs in in vitro models of the liver is the tendency of those cells to spontaneously activate in those culture conditions. As this phenomenon was expected to be related to the difference in microenvironment between *in vivo* and *in vitro* conditions, optimization was focused on that matter. Optimal conditions were found by embedding the cells in a matrix which composition mimicked the healthy liver. This condition differed from conventional culture method as far as collagen type-I, which is found to be secreted by HSCs during activation (Chatterjee et al., 2005) was not included in the culture. While the optimized culture condition led to less expression of Alpha-SMA than in conventional cultures (Fig. 3) activation was still found to be possible after stimulation with inflammatory cytokines such as TGF-Beta and further confirms the relevance of the culture condition and cellular model for further pathophysiological modeling.

Coculture of hiPSCs-derived HSCs, LSECS, and hepatocytes

Coculture of hiPSCs-derived LPCs, HSCs, and LSECs was preliminary performed in both static culture and microfluidic devices. In those trials, coculture was performed in an organized manner. In both culture conditions, the formation of a hepatic tissue, comprised of typical cuboid hepatocytes-like cells was confirmed. Further analysis at the gene level, including cell sequencing are being performed as well as analysis of the cytokine release to fully characterize the model.

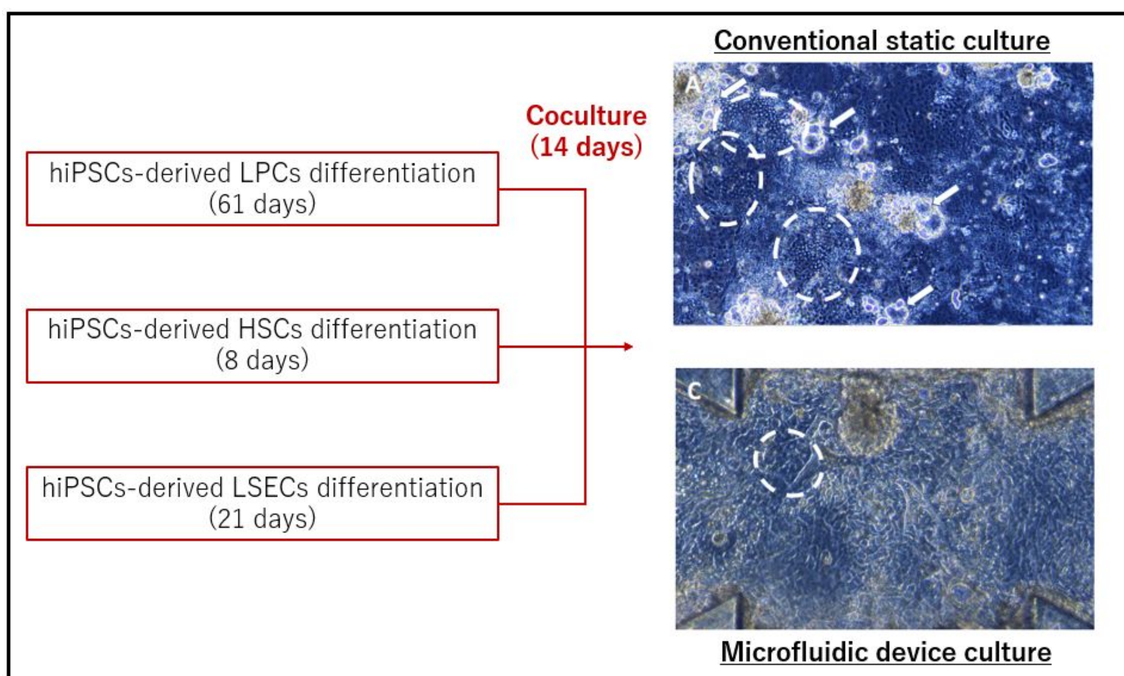

Figure 4: Protocol and preliminary results for the coculture of hiPSCs-derived LPCs, HSCs, and LSECs

Remaining studies

While further analysis is required and is currently performed on the previously described models, better relevancy is currently targeted by building a hierarchical coculture model composed of hepatocytes, hiPSCs-derived HSCs and LSECs. The model is being built taking in consideration the optimization of the HSCs culture previously detailed by including the adequate extracellular matrix for the culture of those cells. After establishment of the model, further inclusion of a micro physiological flow is being considered for further improvement towards the representation of the *in vivo* situation.

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　2件／うちオープンアクセス　2件）

１．著者名	４．巻
Danoy Mathieu、Poulain Stephane、Scheidecker Benedikt、Jellali Rachid、Tauran Yannick、Leduc Marjorie、Bruce Johanna、Gilard Francoise、Gakiere Bertrand、Arakawa Hiroshi、Kato Yukio、Kim Soo Hyeon、Kido Taketomo、Miyajima Atsushi、Sakai Yasuyuki、Leclerc Eric	181
２．論文標題	５．発行年
Influence of CPM-dependent sorting on the multi-omics profile of hepatocyte-like cells matured in microscale biochips	2022年
３．雑誌名	６．最初と最後の頁
Biochemical Engineering Journal	108408～108408
掲載論文のDOI（デジタルオブジェクト識別子）	査読の有無
10.1016/j.bej.2022.108408	有
オープンアクセス	国際共著
オープンアクセスではない、又はオープンアクセスが困難	該当する

１．著者名	４．巻
Danoy Mathieu、Tauran Yannick、Poulain Stephane、Jellali Rachid、Bruce Johanna、Leduc Marjorie、Le Gall Morgane、Koui Yuta、Arakawa Hiroshi、Gilard Francoise、Gakiere Bertrand、Kato Yukio、Plessy Charles、Kido Taketomo、Miyajima Atsushi、Sakai Yasuyuki、Leclerc Eric	5
２．論文標題	５．発行年
Investigation of the hepatic development in the coculture of hiPSCs-derived LSECs and HLCs in a fluidic microenvironment	2021年
３．雑誌名	６．最初と最後の頁
APL Bioengineering	026104～026104
掲載論文のDOI（デジタルオブジェクト識別子）	査読の有無
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オープンアクセス	国際共著
オープンアクセスとしている（また、その予定である）	該当する

１．著者名	４．巻
Danoy Mathieu、Jellali Rachid、Tauran Yannick、Bruce Johanna、Leduc Marjorie、Gilard Fran?oise、Gaki?re Bertrand、Scheidecker Benedikt、Kido Taketomo、Miyajima Atsushi、Soncin Fabrice、Sakai Yasuyuki、Leclerc Eric	120
２．論文標題	５．発行年
Characterization of the proteome and metabolome of human liver sinusoidal endothelial-like cells derived from induced pluripotent stem cells	2021年
３．雑誌名	６．最初と最後の頁
Differentiation	28～35
掲載論文のDOI（デジタルオブジェクト識別子）	査読の有無
10.1016/j.diff.2021.06.001	有
オープンアクセス	国際共著
オープンアクセスとしている（また、その予定である）	－

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　0件）

1．発表者名 Ya Gong , Mathieu Danoy , Taketomo Kido , Masaki Nishikawa , Atsushi Miyajima , Yasuyuki Sakai
2．発表標題 Optimization of In vitro Culture Condition to Maintain Hepatic Stellate Cell in Quiescence
3．学会等名 細胞アッセイ研究会シンポジウム
4．発表年 2021年

〔図書〕　計0件

〔産業財産権〕

〔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