Title:
Generation of Multicellular 3D Liver Assembloids from hiPSC-Derived Liver Organoids and Endothelial Cells: a Proof of Concept.

Authors:
Perriera Riccardo[1], Ferraro Clelia[1], Minjie Zhao[1,3], Cimino Maura[1], Chinnici Cinzia[2], Miceli Vitale[1], Pinzani Massimo[1], Zito Giovanni[1].

Affiliations:
[1]Research Department, Istituto Mediterraneo per i Trapianti e Terapie ad alta specializzazione – IRCCS ISMETT, Via Ernesto Tricomi 5, Palermo, Italy.
[2]Ri.MED Foundation, via Ernesto Tricomi 5, Palermo, Italy.
[3]Department of Hepatobiliary Surgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China.

Background and Aim:
Traditional 2D liver cell cultures fail to recapitulate the structural and functional complexity of the in vivo liver microenvironment [1]. In contrast, three-dimensional (3D) models - particularly assembloids that integrate multiple cell types - provide a more physiologically relevant platform to investigate liver functions and disease mechanisms [2]. Human induced pluripotent stem cells (hiPSCs) offer reproducibility, scalability, and the ability to self-organize into functional liver organoids, positioning them as an attractive source for assembling 3D liver models [3]. In this proof-of-concept study, we aimed to generate multicellular 3D liver assembloids by combining hiPSC-derived liver organoids (hiPSC-LOs) with human umbilical vein endothelial cells (HUVECs).

Methods:
hiPSCs were differentiated into hepatocyte using the STEMdiff™ Hepatocyte Kit under adherent and non-adherent conditions to generate 2D hiPSC-hepatocytes (hiPSC-HEPs) and 3D hiPSC-LOs, respectively. Hepatocyte identity and function were assessed by marker expression and albumin release. Multicellular liver assembloids were generated by combining 3D hiPSC-LOs with HUVECs at defined ratios and characterized using IF and whole-mount staining for hepatocyte and endothelial markers.

Results:
Functional 2D hiPSC-HEPs were generated, displaying hepatocyte-specific markers and albumin secretion. An optimized protocol yielded 3D hiPSC-LOs with comparable identity. When combined with HUVECs, hiPSC-LOs self-organized into multicellular assembloiuds over 14 days. IF and whole-mount staining confirmed the identity of hepatocytes (CK18+) and endothelial cells (CD31+), with HUVECs forming elongated, organized structures around hepatocytes, resembling in vivo endothelial organization.

Conclusions:
We demonstrate the feasibility of generating functional hiPSC-LOs and multicellular 3D liver assembloids with endothelial cells. Although protocols for liver sinusoidal endothelial cells (LSECs) and Kupffer cells are under development, these preliminary results highlight the potential of 3D assembloids to model the liver niche and study cellular interactions in health and disease.

Bibliography:

1. Urzì O, et al. PMID: 37569426.
2. Kim Y, et al. PMID: 39722609.
3. Koui Y, et al. PMID: 40782899.