3D bioengineered liver for the study of acute and chronic hepatic damage

Ainhoa Ferret-Miñana[1], Estefanía Alcaraz[2], Raquel Horrillo[2], Javier Ramón-Azcón[1,3], and Francesco De Chiara[1]

[1]*Biosensors for Bioengineering Group, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain*
[2]*Scientific Innovation Office, Grifols, Barcelona, Spain*
[3]*ICREA-Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain*

Background

Acute and chronic liver damage are significant health conditions. Acute liver damage refers to sudden and severe injury, often caused by drug toxicity. Chronic liver damage, on the other hand, involves long-term injury to the liver caused by many conditions. One of them is the intake of some drugs for a prolonged time. Fibrosis, a hallmark of liver damage, is primarily caused by the activation of hepatic stellate cells (HSCs). Understanding the mechanisms underlying acute and chronic liver damage is crucial for developing effective treatments. Conventional liver models have many limitations. 2D cultures lose liver phenotype and functions quickly, hindering chronic exposure modelling. Moreover, reproducing fibrosis in 2D cultures becomes challenging due to the activation of HSCs on plastic or glass surfaces. Thus, 3D models are a valuable tool for studying these diseases, allowing to investigate disease progression, identify potential therapeutic targets, and develop new drugs. Here, we present our 3D models for both chronic and acute liver diseases, illustrating the evolving disease phenotype as it advances over time.

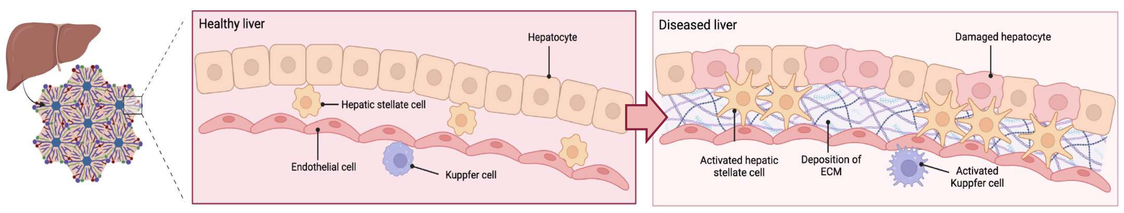

Figure 1. Schematic representation of a healthy and a diseased liver.

Methodology

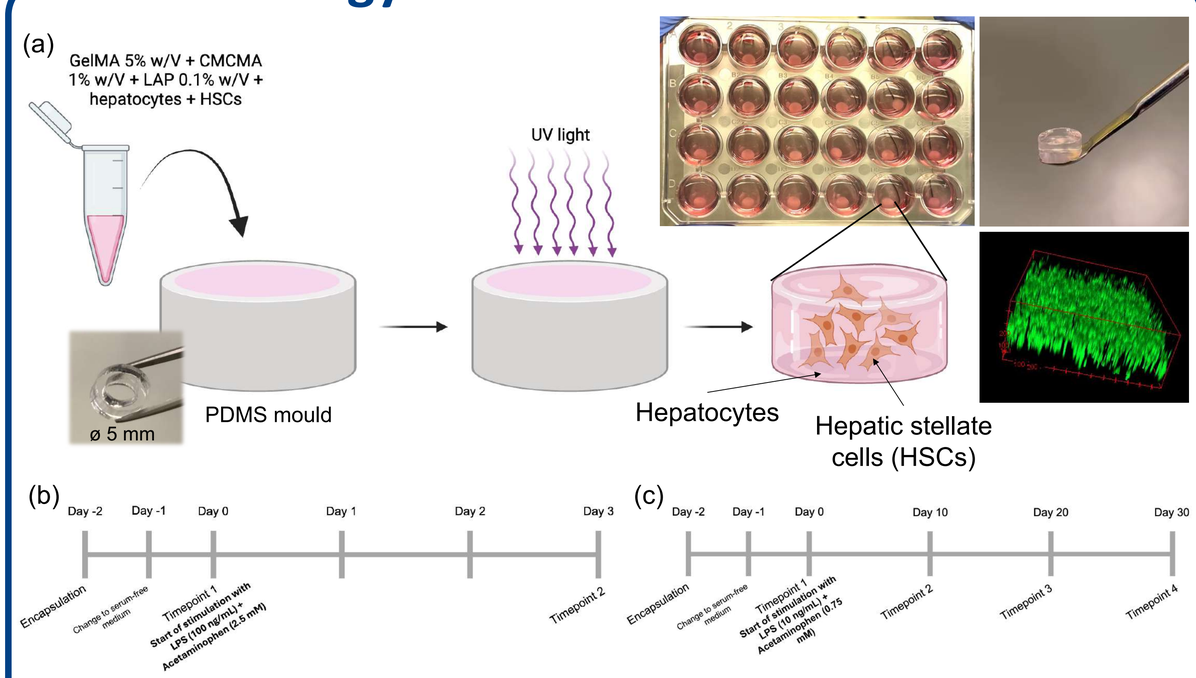

Figure 2. Fabrication of the 3D human liver tissues. (a) The hepatocytes and hepatic stellate cells are encapsulated in a prepolymer solution of GelMA-CMCMA to fabricate the liver tissues. LAP 0,1% w/V is used as a photoinitiator. Experimental plan of the acute (b) and chronic damage (c) models.

Gene Expression Profiling in 3D Livers

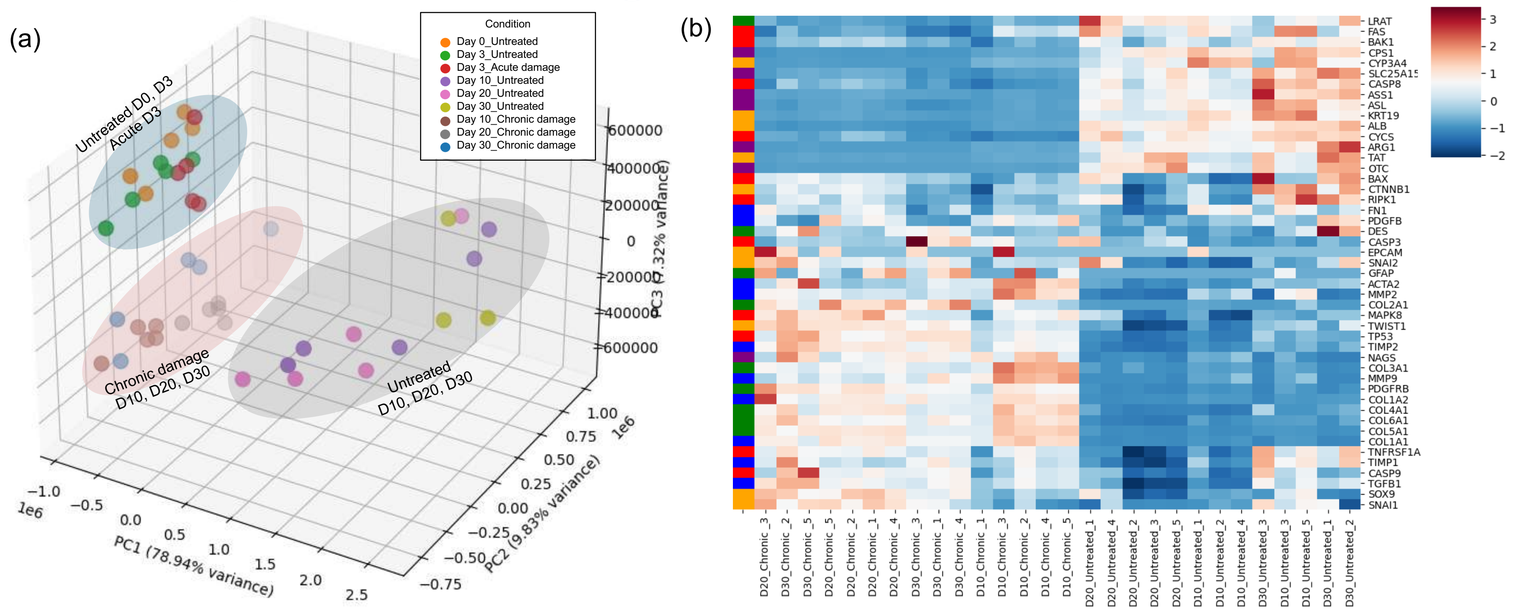

Figure 3. RNA-Seq analysis of the 3D human liver tissues. (a) Principal Component Analysis plot. It shows the clustering of samples based on the global gene expression profiles under different experimental conditions: untreated, acute damage, and chronic damage over time (Day 0, Day 3, Day 10, Day 20, Day 30). (b) Heatmap compares the expression of genes across different biological processes between untreated and chronic damage conditions over time. The genes are grouped by biological processes such as Fibrosis, Hepatic Stellate Cell Activation, Hepatocyte Death, Urea Cycle, and Hepatocyte Differentiation.

Acute Liver Damage Model

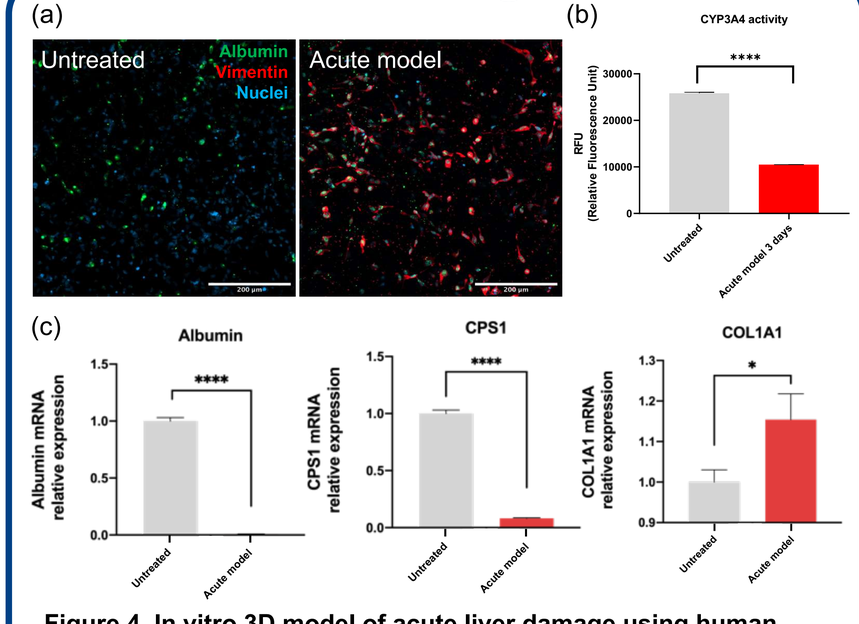

Figure 4. In vitro 3D model of acute liver damage using human hepatocytes and HSCs. (a) Confocal images of the 3D co-cultures of a hepatocyte marker (albumin) and a hepatic stellate cell activation marker (vimentin). (b) Cytochrome assay (CYP3A4). (c) qPCR analysis of hepatocyte's functionality markers (albumin and CPS1) and an HSC's activation marker (COL1A1).

Chronic Liver Damage Model

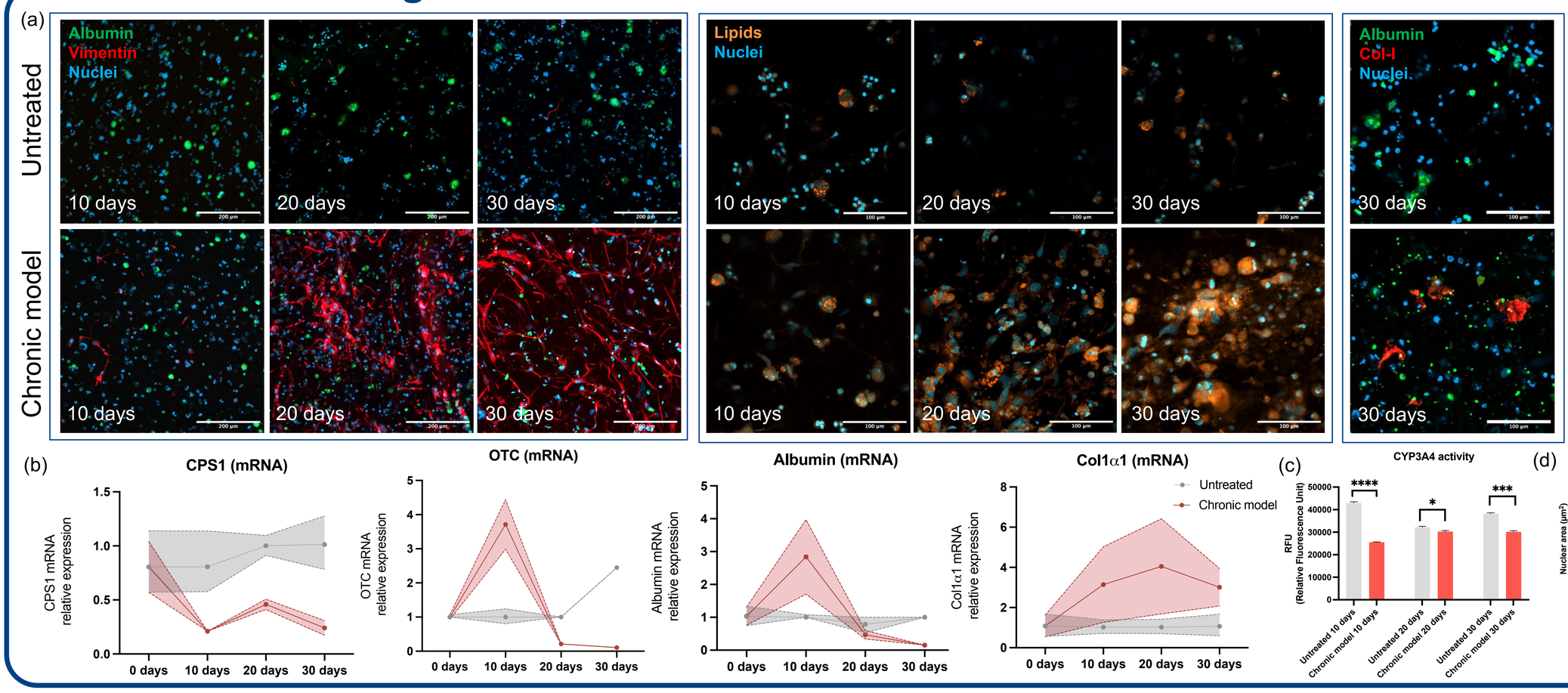

Figure 3. In vitro three-dimensional model of chronic liver damage using human hepatocytes and hepatic stellate cells. (a) Confocal images of the 3D co-cultures of a hepatocyte marker (albumin) and hepatic stellate cell activation markers (vimentin and collagen Type-1), lipid accumulation, and nuclei. (b) qPCR analysis of hepatocyte's functionality markers (albumin, and OTC and CPS1) and an HSC's activation marker (COL1A1). (c) Cytochrome assay (CYP3A4). (d) Quantification of the nuclear area.

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