nature research

https://doi.org/10.1038/s41575-020-00386-1

Supplementary information

Human biomimetic liver microphysiology systems in drug development and precision medicine

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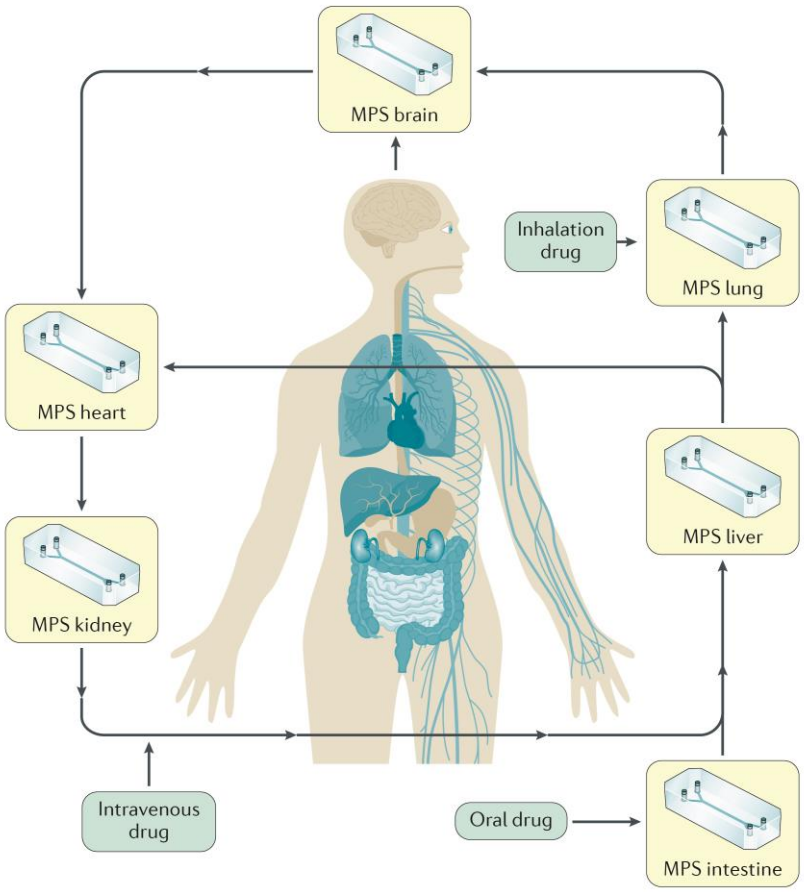

Supplementary Figure 1. Example of a multi-organ MPS. All integrated multi-organ systems would adhere to known human physiology. In this example, 4 elimination organs (intestine, liver, kidney and lung); 2 barrier layers (intestine, blood brain barrier) and the heart are coupled. 3-drug insertion points are illustrated which demonstrate how this configuration could be used for ADMET. A similar configuration using organ MPS for intestine, liver, pancreas and white adipose tissue would be an integrated multi-organ platform to study complex liver diseases such as NAFLD/NASH and T2DM.

Supplementary Table 1. Heat Map of the Comparative and Complementary Characteristics of Human Liver Epithelial Organoids, Current human biomimetic-MPS (HBL-MPS), and Next Generation Structured-MPS and Organoid-MPS (Green indicates ability to fulfill the objective and red indicates that the objective cannot be met).

Characteristics	Epithelial Organoids	Current HBL-MPS	Next Generation: Structured-MPS & Organoid MPS-MPS
Data Acquisition			
Real-Time Imaging Metrics			
In-Line Sensors of Functions			
Temporal and Spatial Content of Read-outs			
Controlling the microphysiological state			
Fluidic and Mechanical Cues			
Access to Microbiome			
Control of Microenvironment (e.g. Zonation, Matrix)			
Control of Immune Cell Infiltration			
Genetic Control of Cell state			
Cell types and non-epithelial components			
Use of Stem Cells			
Renewable cell types			
Presence of native Cell Types			
Innervation			
Vascularization			
Extent of Recapitulating Human Liver Acinus Structure			
Throughput and heterogeneity			
Homogeneity of individual models			
Ease of Production			
Throughput of studies			
Breadth of Applications in Biomedicine			
Key to Characteristics: ■-Missing, ■-Partial, ■- Included			

Supplementary Table 2. Required Functions of Adult, PSC-derived Liver Tissue in HBL-MPS for Physiological & Clinically Relevant Applications. [1-5]

Functional characteristics	Description
Active transport proteins	NTCP, OATPs, OAT, OCTs, MDR, BSEP, BCR, MRPs
Expression and activity of clinically relevant enzymes	UGT1A1, G-6-Pase, A1AT, OTC, (ATP7B) copper-transporting ATPase 2, (PAH gene) enzyme phenylalanine hydroxylase, branched-chain alpha-keto acid dehydrogenase complex (BCKDC), FAH
Drug Metabolism (Cytochrome activity)	(CYP450) CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP7A1
Expression and activity of xenosensors	Ahr, PXR, CAR, XRE,
Expression and transcription of liver specific transcription factors	HNF4a (adult isoform), CEBPa, CEBPb, HNF1a,
Hepatic synthetic functions	Secretion of albumin, A1AT, fibronectin, apoproteins, transthyretin, complement factors, coagulation factors (II, V, VII, IX, X), synthesis of Urea,
Other important metabolic function	Synthesis of cholic acid and chenodeoxycholic acid, Formation of 7α-hydroxycholesterol,
Absence of markers associated with fetal stage	LGR5, OCT4, CD133, AFP, CYP3A7
Morphological features of liver cellular complexity	Liver tissue tight junctions (Claudin-1, 10, occluding, ZO1), liver specific gap junctions (e.g. Cx32, Cx26, Cx43)
Bile duct (cholangiocytes) functions	Transport functions, CFTR (Cl- secretion), ASBT (bile acid absorption), SGLT1 (glucose and amino acids absorption), AQP1 (water channel), secretin-induced release of bicarbonate, AE2 (Cl- / bicarbonate exchanger)
Liver sinusoidal endothelial cell system functions [215]	CD4, CD32, ICAM1, Fenestrae, blood flow regulation, permeable barrier of different molecules, endocytosis, immune modulators
Functions of liver sinusoids	oxygen and biochemical zonation

Supplemental Table 3: Example phenotypic, functional and genomic metrics of biomimetic MPS for liver metabolic syndrome disease models

Readout	MPS Live Measurements	Clinical Relevance
Secretome		
Albumin secretion	ELISA[7]	Metabolic competence, overall hepatocyte function
Urea secretion	Colorimetric[7]	Metabolic competence, overall hepatocyte function
LDH release[+]	Colorimetric[7]	Hepatocellular injury corresponding with ALT and AST
Glucose secretion	AmplexRed Glucose Assay[8]	Glucose regulation
β-hydroxybutyrate	Colorimetric[9]	Fatty acid oxidation
Lactate secretion	Colorimetric[10]	Glycolysis
Cytokine release	Human Cytokine Panel[11]	Liver stress/injury response
Fatty acid secretion	Mass spectroscopy[12]	Fatty acid metabolism
TNFα secretion	ELISA[7]	Kupffer activation, innate inflammatory response
Bile acids (BA)	BA profile by Mass spectroscopy[13]	NAFLD, NASH altered plasma BA profile corresponds
Exosomes (proteins/RNA/microRNA)	antibody (α-SK1), qRT-PCR[14]	Circulating extracellular vesicles containing SK1, miRNA-122 & -21 as markers for NAFLD, NASH, HCC
Biosensors		
Hepatocyte Apoptosis	Biosensor[7,15]	Hepatocellular injury/death
ROS (Hepatocytes & Kupffer)	Biosensor[7,15]	Hepatocellular injury, Kupffer cell activation
DAG (Hepatocytes)	Biosensor[16]	Insulin resistance marker
ER stress/UPR Hepatocytes	Biosensor[16]	Hepatocellular damage
Insulin resistance (Hepatocytes)	Biosensor[17,18]	Lost suppression of glucose production
Cell Tracking (stellate & Kupffer)	Biosensor[15]	Morphology of early liver injury, proliferation
JNK activity	Biosensor[19]	Contributes to inflammation, fibrosis, cancer growth, and metabolic diseases of the liver
Imaging Measures		
Steatosis	Brightfield & LipidTox[20]	Normal and/or pathological fat storage in hepatocytes
PMN Infiltration	Imaging labeled PMNs[21]	Adaptive inflammatory response
Mitochondrial Function	TMRE[20]	Hepatocellular health and function
Glucose Uptake	6-NBDG[22]	Hepatocellular insulin resistance marker
Endpoint Measurements		
Glycogen	Antibody[23] or PAS stain[24]	Glucose regulation
Hepatocyte Ballooning	Cytokeratin, anti-CK8/18	A key indication of NASH[25]
LSEC Activation	ICAM antibody – IF[26]	Regulates fibrosis and inflammation responses
Fibrosis (Stellate Activation & Collagen Synthesis)	α-SMA, COL1A2 IF[7]	Early fibrosis indicator and direct measure of fibrosis
RNAseq	RNA sequencing, multiple methods[27]	Comparison to patient results[28,29]

*Based on known clinical data; α-SK1 = sphingosine kinase 1 ; α-SMA = alpha smooth muscle actin; COL1A2 = collagen type 1, alpha 2; 6-NBDG- fluorescent glucose probe; DAG diacylglycerol

Supplementary Table 4: Examples of potential gene-based or secreted clinically relevant biomarkers in HBL-MPS[30-37]

Disease	Relevant genes			Secreted molecule(s)
	Variant	**Transcription factor**	**Other**	
NAFLD (Steatosis vs NASH)	PNPLA3 MBOAT7 GCKR TM6SF2 TCF7L2	FXR HNF4a SREBP CEBPa PPARa		cytokeratin 18 caspase-cleaved fragments, IL-6, adiponectin, TNFa, IL-8, pyroglutamate, Fibroblast growth factor (FGF) 21 in combination with cytokeratin 18 cleavage fragments, miR-34a, miR-122, miR-192, miR-21, miR-375, miR-16, miR-222
Fibrosis	PNPLA3 MBOAT7 GCKR TM6SF2 TCF7L2	SMAD3 STAT3 YAP1 GLI2 AP-1 SOX9 MRTF-A	MMPs TIMPs	Procollagen Type III (PIIINP), precursor C3-protein (PRO-C3), hyaluronic acid, TIMP-1 circulating DNA methylation of PPARg promoter
Alpha1 Antitrypsin Deficiency	**Genetic variants of SERPINA1**			Alpha1 Antitrypsin
	c.187C > T c.194C > T c.226_228delTTC c.230C > T c.551_552delC c.647G > T c.721A > T	c.739C > T c.839A > T c.863A > T c.1096G > A c.1130_1131insT c.1156_1157insC c.1178C > T		
Phenylketonuria	>950 phenylalanine hydroxylase (PAH) gene variants. Most common variants (c.1222C>T, c.1066-11G>A) (23% and 6% of all mutations, respectively)			phenylalanine (Phe)

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