Human Models for improved prediction of DILI

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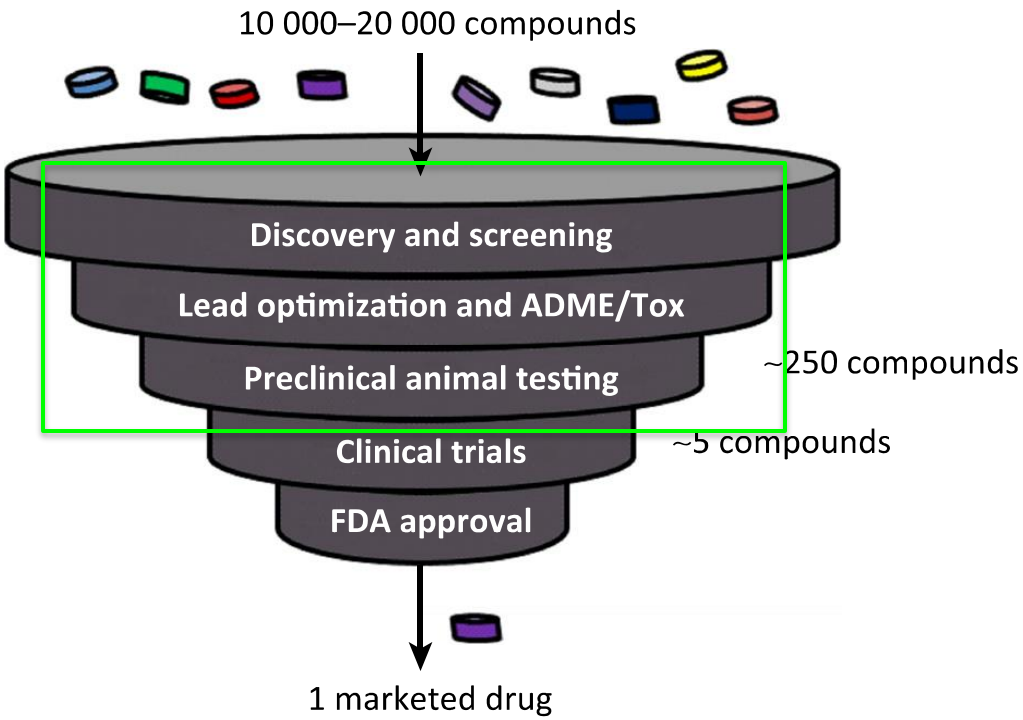
Human Models for improved prediction of DILI - considerations

- The problem
- BioSystems
- Tissue modelling approaches
- Phenotypic prifiling
- HepaRG as a surrogate for PHHs
- Synthetic Biological Engineering
- Technology convergence

The Drug Development Pipeline

Intervention stage(s) for improved human in vitro models



Trends in Biotechnology

Defining the Problem

- R&D Costs: AstraZeneca **$9.7 billion;** Novartis **$6.1b** *[Forbes Mag. 2013]*
- Clinical trial failures/ High drug attrition rates = DILI

Bottlenecks

- Limited predictive power of pre-clinical evaluation methods
- Animal species not predictive of human DILI
- Lack of suitable in vitro human hepatic models

Requirements of *in vitro* Human liver models

- Recapitulate organotypic features – considerations:
  - Cell choice | Biomaterials | Bioreactor | Trophic support
- Physiologically-relevant surrogates to primary human hepatocytes
  - iPSC-derived hepatic cells | HepaRG progenitor cells
- Sustainable/ reproducible/ longevity
- Maintain multiple axes of liver metabolism and function
- Amenable to HCS/ HTS - *and* emerging non-invasive/ analytical techniques

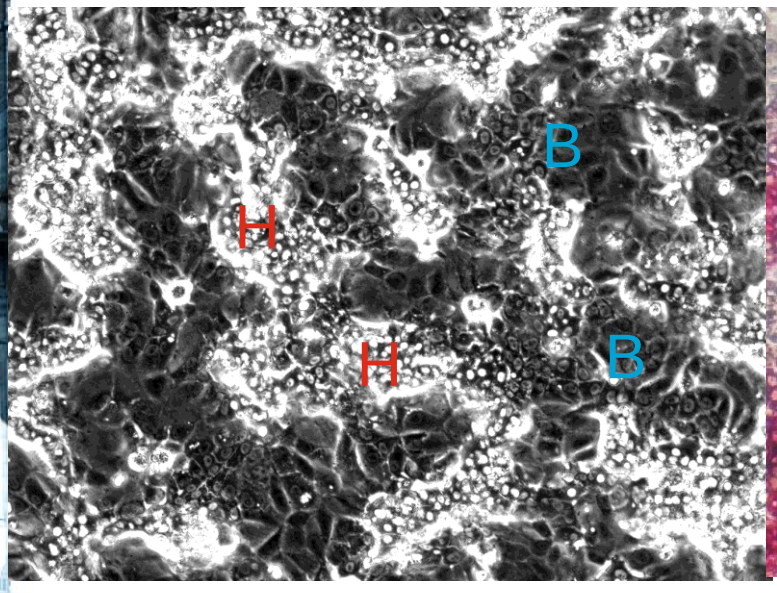
Limitations of existing pre-clinical *in vitro* hepatic models

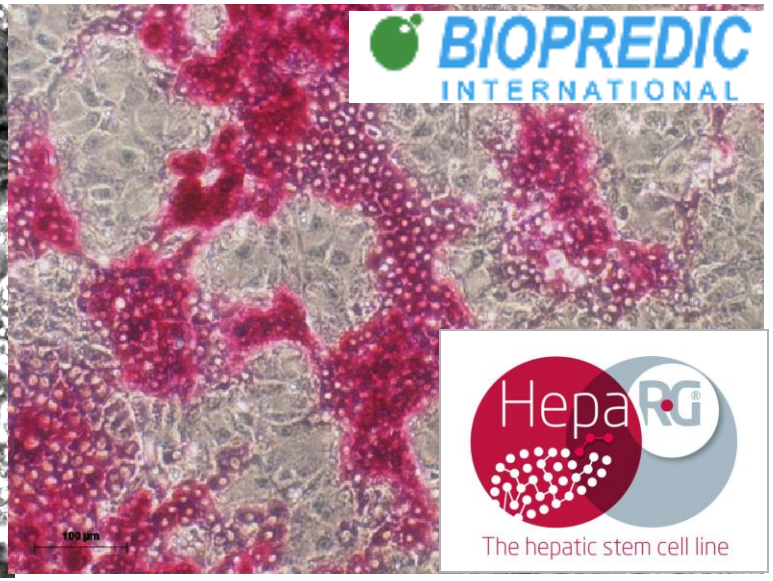
- 2D mono-cultures – fried-egg morphology
  - ➢ Human Hepatic Cell Lines (eg HepG2)
    - Low functional repertoire (eg CYP450s)
    - 'Non-physiological'
- Primary Human Hepatocytes (PHHs)
  - Scarcity/ cost/ variability/ instability
- Rodent primary hepatocytes *(in vivo* also*)*
  - Species differences in drug metabolism (CYP450 isoforms), drug targets, and pathophysiology
- Human iPSC-derived Hepatocyte-like cells
  - Foetal phenotype/ Epigenetic variability

Alternative: Human Liver HepaRG Cells

Potentially suitable **surrogate** to primary human hepatocytes

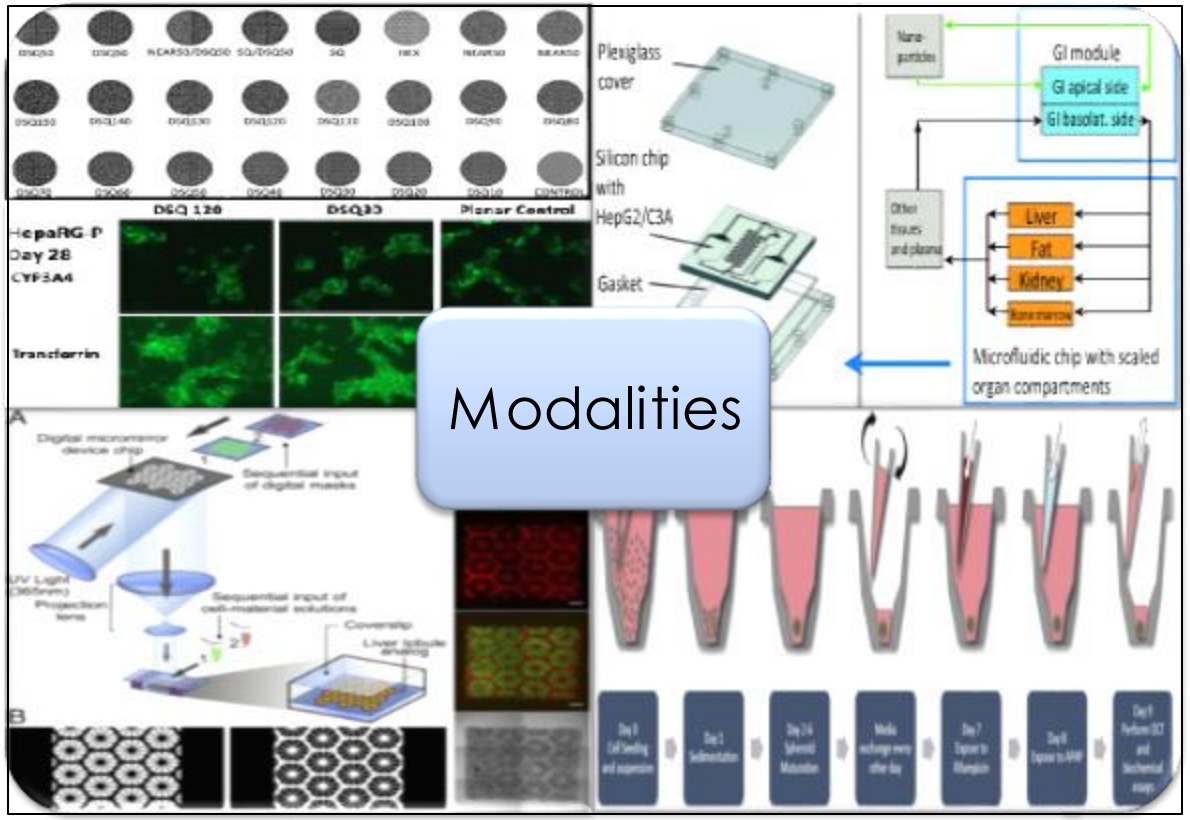
- Bipotent hepatic progenitor signature
  - ✓ Biliary or Hepatocytic lineages
  - ✓ Hepatocyte:Cholangiocyte *intrinsic* co-culture
    - ✓ Stable & Reproducible; Culture longevity >28 days
  - ✓ Differential expression patterns of many genes
    - ✓ Enhanced cell-cell/ cell-matrix interactions
  - ✓ Intact phenotypic functionality





Biosystems for modeling human liver

Directed nanopatterning Microfluidics '*Homo Chipien*'

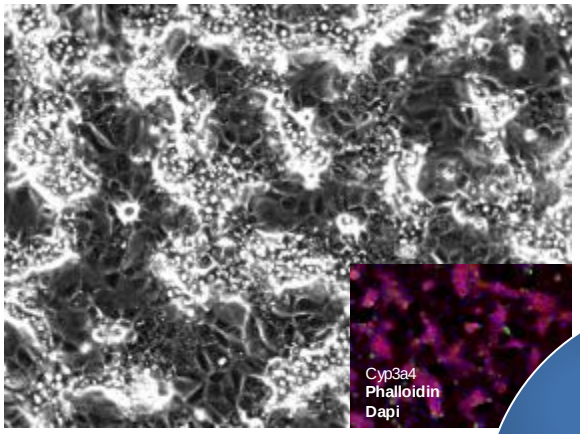


3D bioprinting
(hydrogel based)

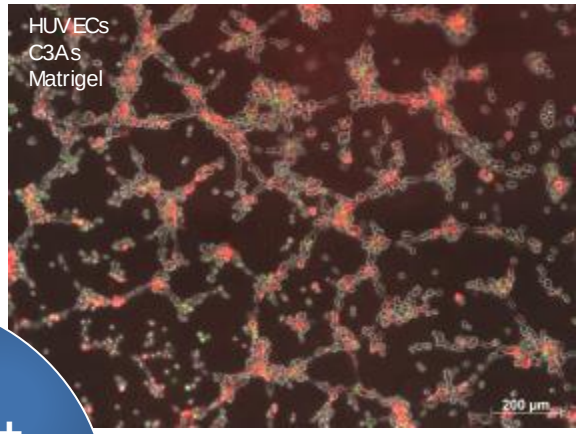
3D liver constructs:
Spheroids | Organoids

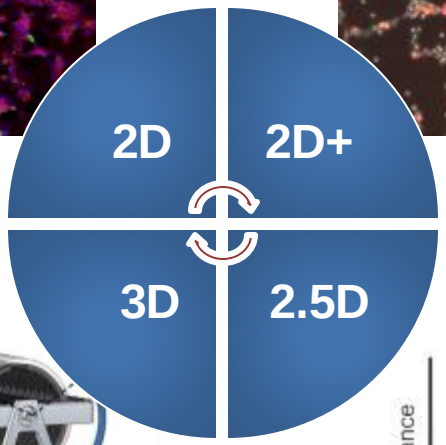
Tissue Modeling Strategies

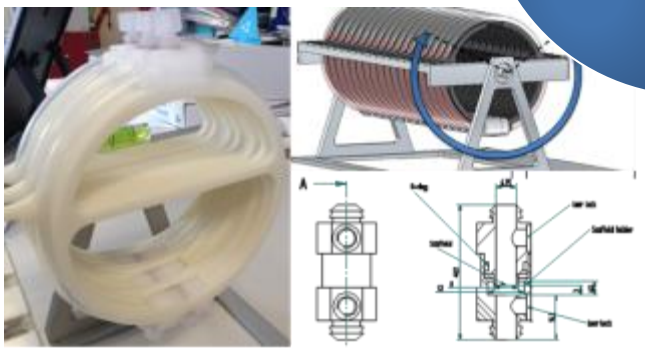
Human HepaRG cell co-culture



Human hepatic and endothelial cell co-culture



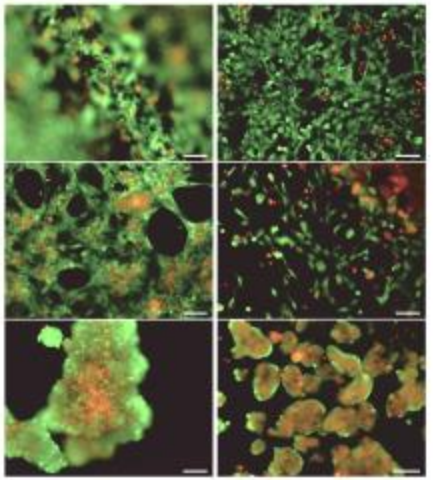




Oscillatory perfusion bioreactor

Overlay Encapsulation

Increased compliance



Liver-like Modulus

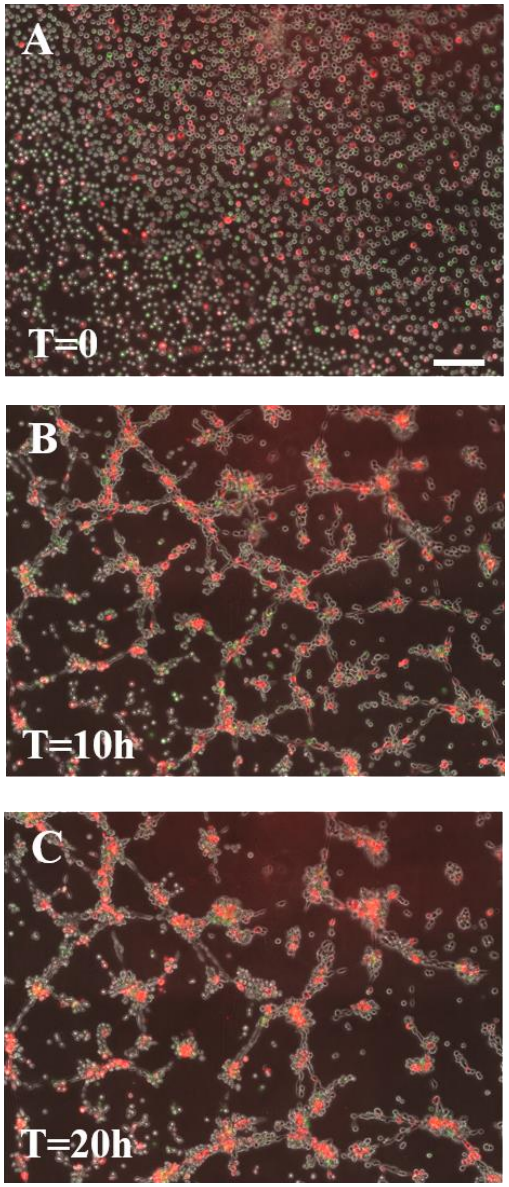
Oscillatory Perfusion Bioreactor [MIT]

Recapitulates physiological interstitial flow

Live/ **Dead** Staining

Hyaluronin-based Hydrogels

Acetaminophen cytotoxicity is ameliorated in a human liver organotypic co-culture model



Time-lapse/ fluorescence microscopy of HUVEC:C3A on Matrigel at times:

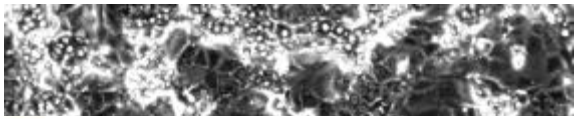
0-24 hours

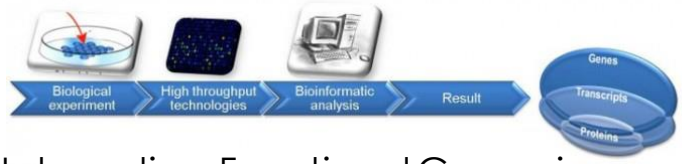
in EGM-2 media

- In C3A:HUVEC co-culture → cells were less **susceptible** to the toxic effects of APAP:
  - **HUVECs>C3As>co-culture**
  - Bidirectional communication/stabilization between different cell types
  - Test candidate compounds differentially targeting hepatocytes and endothelial cells

Phenotypic Profiling: Assay approaches/ Data integration

Human HepaRG cell co-culture





Integration: Functional Genomics

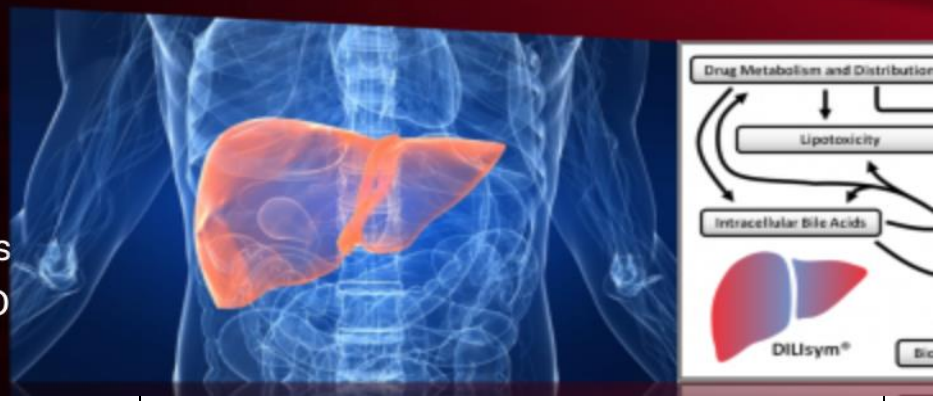
Assays

SimulationsPlus

SCIENCE + SOFTWARE = SUCCESS

DILIsym®

Quantitative systems toxicology (QST) s
for modeling drug-induced liver injury (D



- ATP/ LDH/MTT/ BrdU
- Histology
- Mechanistic studies
  - GSH/ ROS/ Glucose/ Ca^{2+}

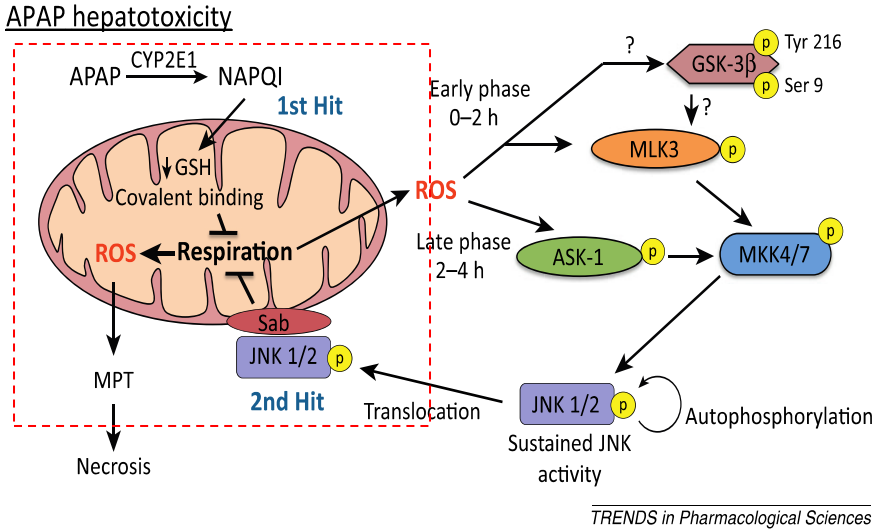
- ECIS

Operetta High-Content Imaging System

Intrinsic DILI: Acetaminophen Hepatotoxicity

Classic acetaminophen hepatotoxicity studies

Biochemical endpoint assays



Histopathology (mouse liver tissue)

ATP depletion
Mitochondrial toxicity
Glutathione content
etc

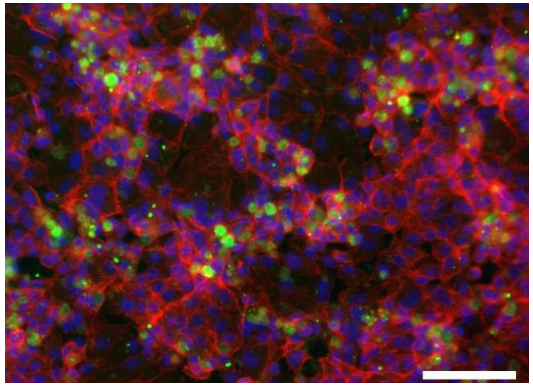
Barrier function eg
TEER:
Tight junction protein
expression

Centrilobular necrosis

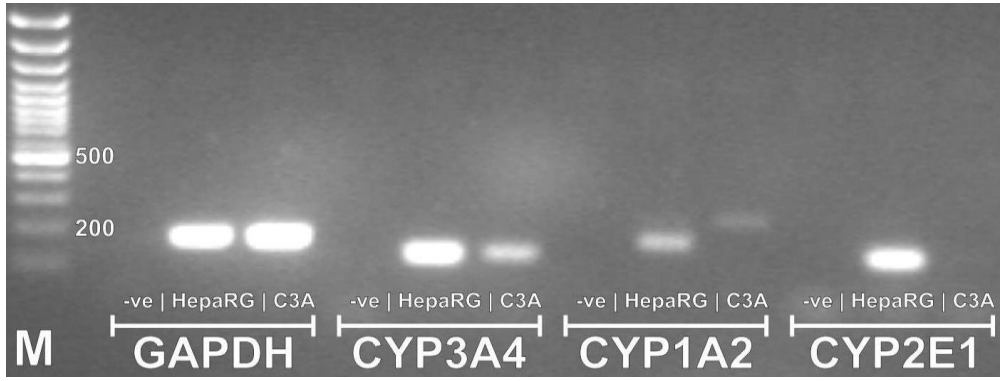
Invasive/ destructive/
time-consuming…

Non-invasive methods in Liver Toxicity: Acetaminophen-disruption of liver architecture

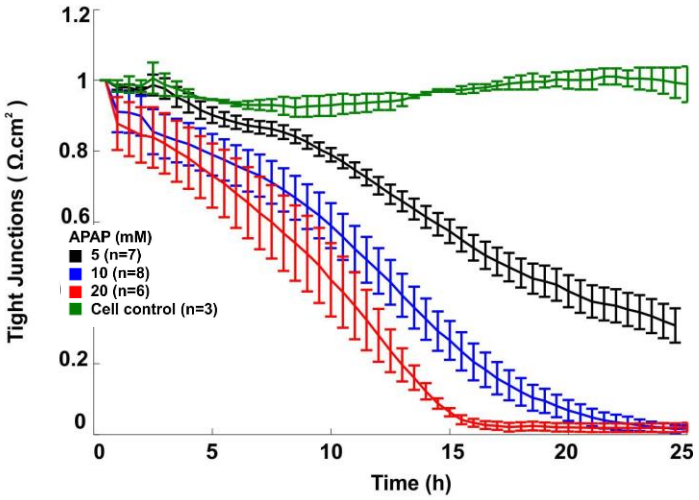
HepaRG differentiated phenotype



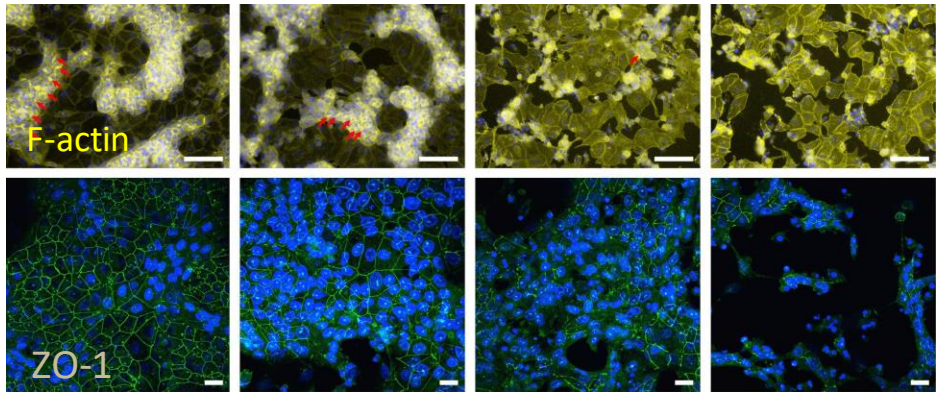
F-actin/CYP3A4/DAPI



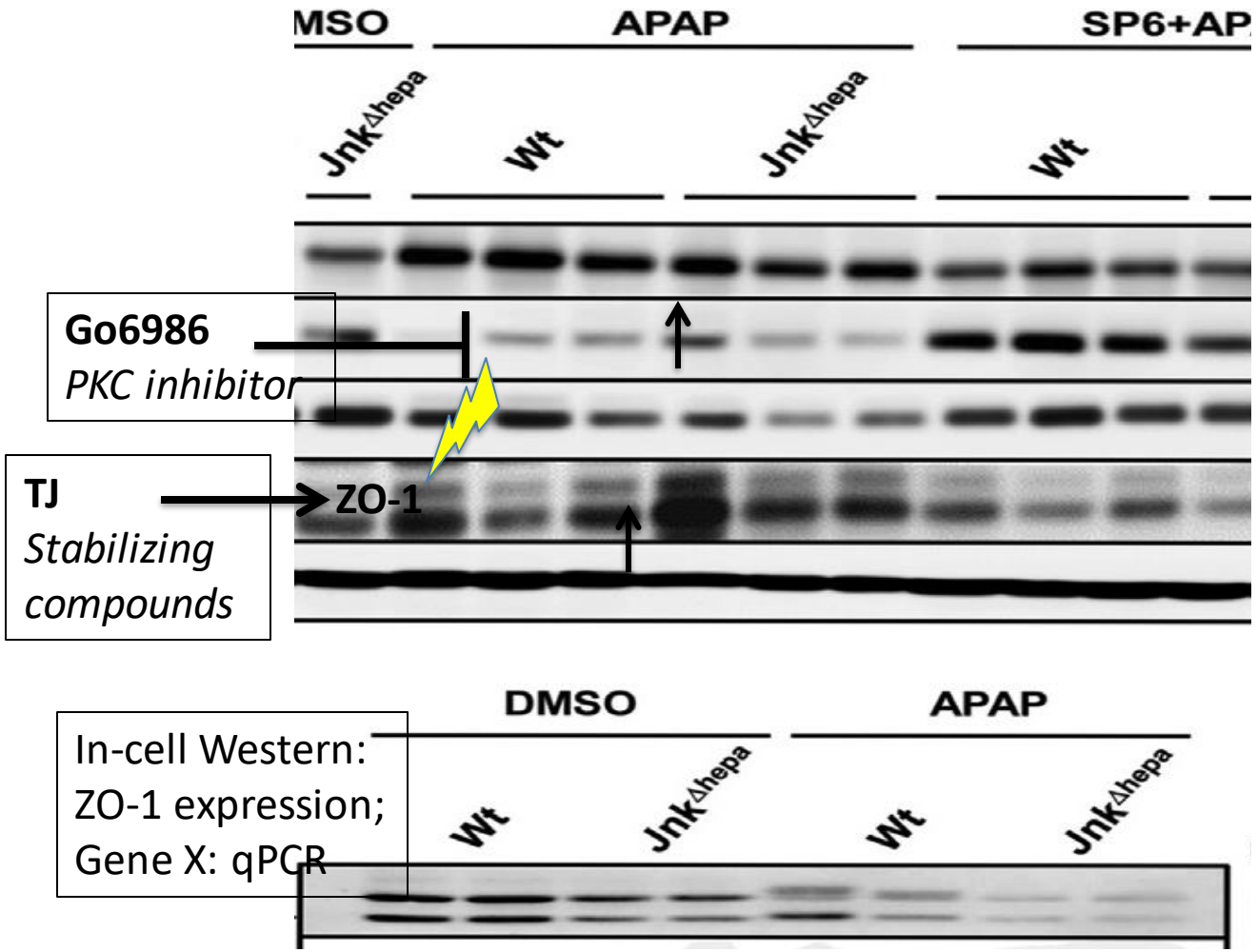
ECIS: Tight Junctions



Structural disruption: F-actin/ Tight Junctions



Mechanistic insights following ECIS



Adapted from: Cubero et al., Gastroenterology 2016;150:968–981

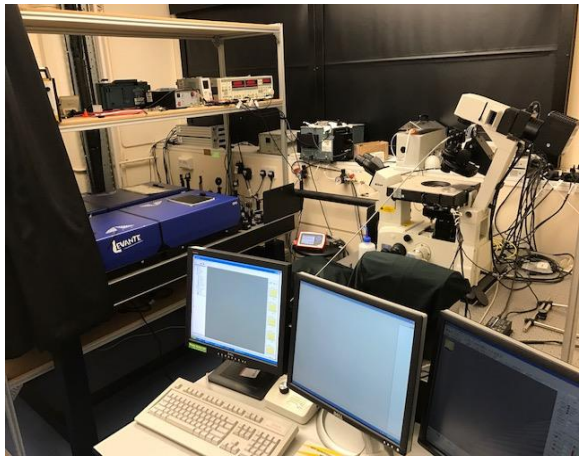
Non-invasive quantitative imaging - CARS label-free imaging of lipids

2D-HepaRG cells – fat loading (NAFLD) model
LPON/ Oleate Feed for 72 hours

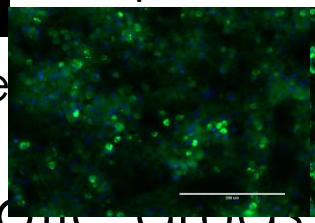
LPON

Oleate

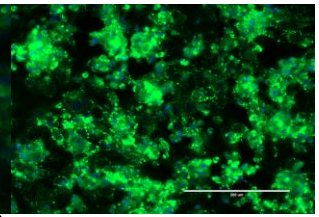
CARS: Coherent Anti-Stokes Raman Scatte



HepaRG cells vs BODIPY



Control



LPON

Can be applied to DILI eg steatotic drugs

Commercially-available 3D culture systems

Company	Culture system	Comments
InSphero	'Hanging drop' spheroids; or microtissues	✗ Scaffold
Cyprotex	HepaRG-spheroid	✗ Scaffold
Hepregen	'Liver microtissues'	✓ Scaffold ✗ Sustainable (PHHs; animal components)
BIOMIMESYS®	Modular/hydrogel-scaffold	✓ Biocompatible / multiple cell types

✓ Ideal characteristics

That better mimic *in vivo* liver physiology

Features:

- Create cellular interactions
- Greater structural complexity
- Enabling more accurate and earlier predictions of drug toxicity – save £m

- ✓ Scaffold + Cells = organotypic
- ✓ Animal-free components
- ✓ Supply plated 'Ready-to-go' culture system
- ✓ Modular = add other cell types
- ✓ 'Plug-in' to existing tox testing regime

HCS Pharma

BIOMIMESYS®
natural HA-based hydroscaffold

The BIOMIMESYS® products are formed by a crosslinking reaction of hydrosoluble modified hyaluronic acid and other extracellular matrix components (ECM) with ADH (Adipic acid dihydrazide):

Per organ

Coming soon
BioMIMESYS® Brain

HA + RGDS + Galactose + Collagen I & IV
E = 0.6 kPa
BioMIMESYS® Liver

HA + RGDS + Collagen I & VI
E = 0.45 kPa
BioMIMESYS® Adipose Tissue

Per pathology

HA + Collagen I
E = 1 kPa
BioMIMESYS® Oncology
Different E for different stages: ongoing

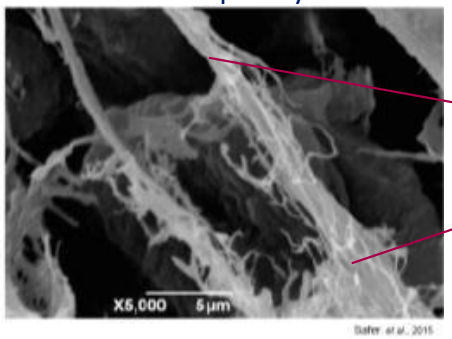
Coming soon
BioMIMESYS® NASH
BioMIMESYS® Liver Fibrosis

HCS Pharma

BIOMIMESYS® *Liver*

IN VIVO

Decellularized hepatocyte ECM

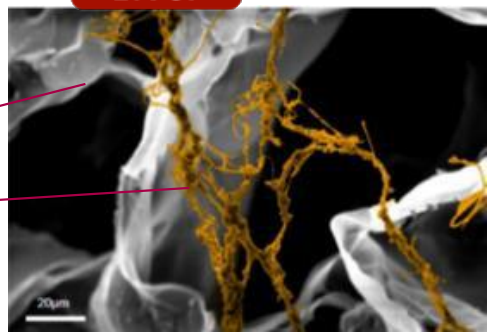


Hyaluronic sheets

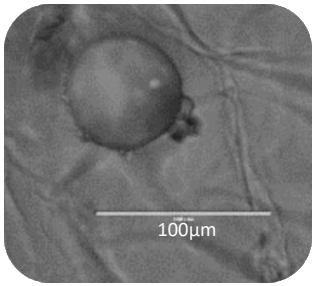
Fiber of collagens

BioMIMESYS® Liver

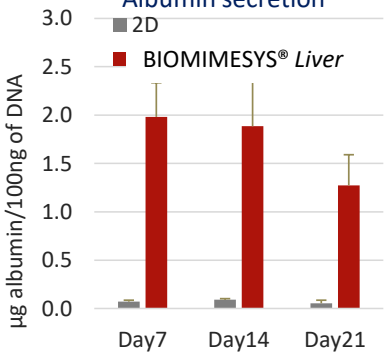
IN VITRO



Human hepatocytes

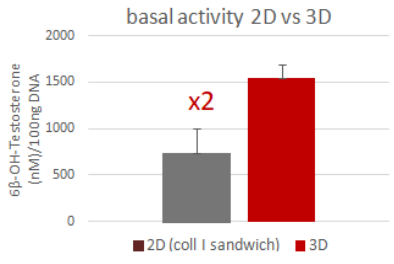


Albumin secretion

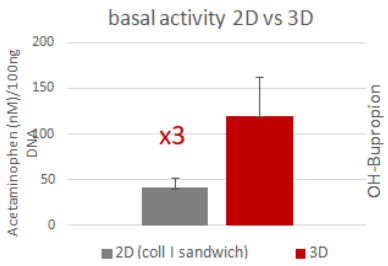


& CYP activity in primary human hepatocytes

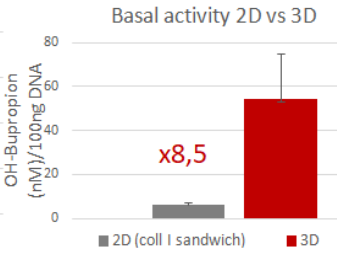
CYP3A4



CYP1A1/1A2



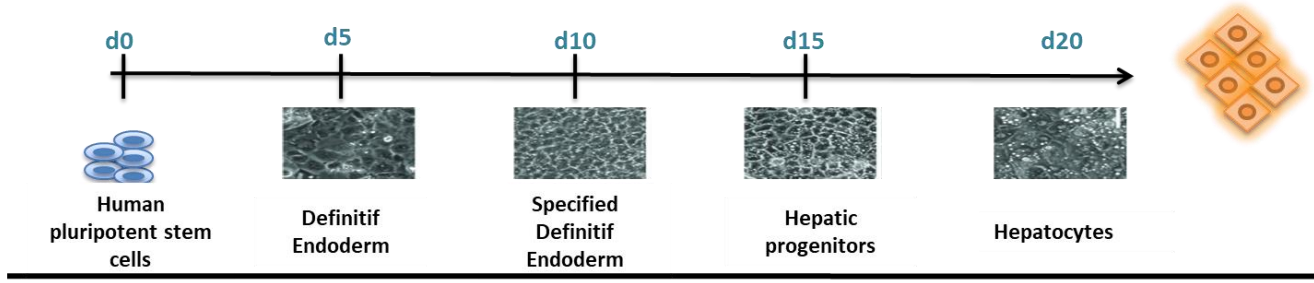
CYP2B6

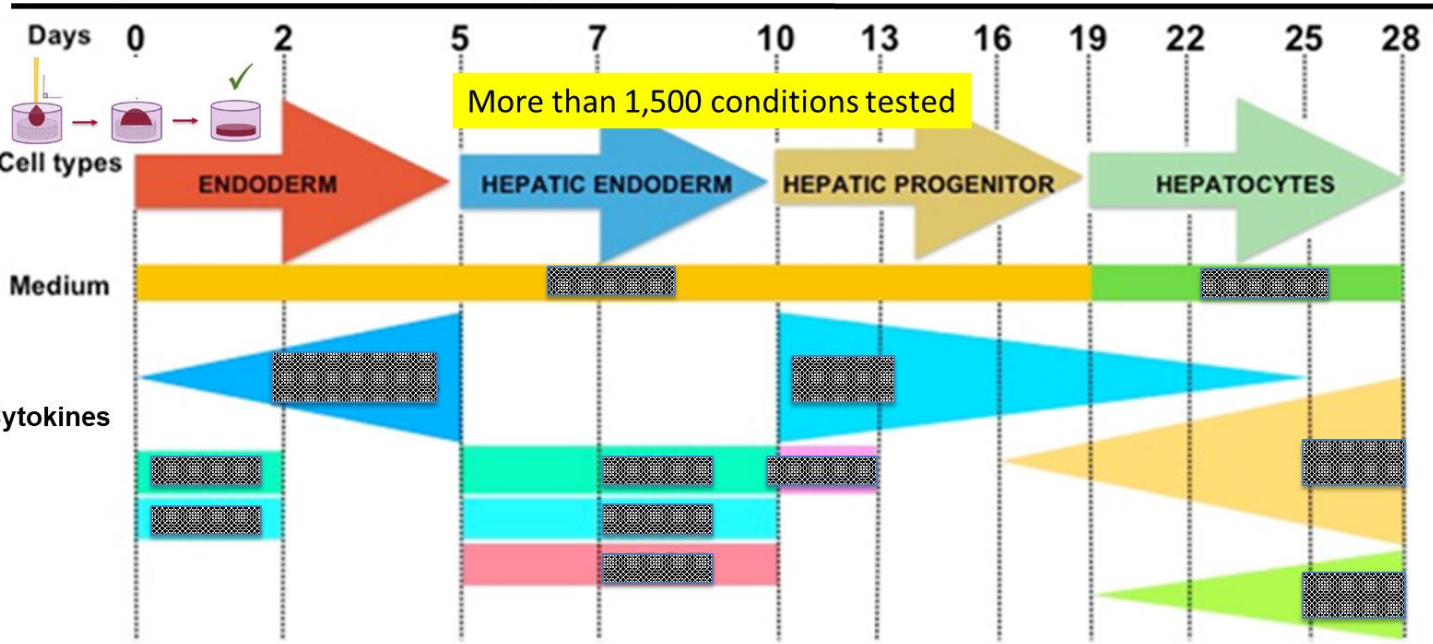


Human hepatocytes keep their functionnality until 1 month!!! => chronic assay possible

Human iPSC-differentiation → Hepatocyte-like cells

New differentiation protocol for iPSCs in BIOMIMESYS®





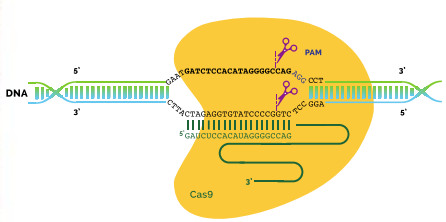
* : Patent in preparation

Novel *in* vitro human liver model for predicting DILI

SYNTHEGO

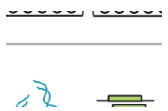
BIOPREDIC INTERNATIONAL

Academia-Industry Synergy: Synthetic Biological Engineering



POR gene

KO



Genotype/ Phenotypic

Functional Profiling

Synthego CRISPRevolution System

Biopredic Human liver HepaRG cells

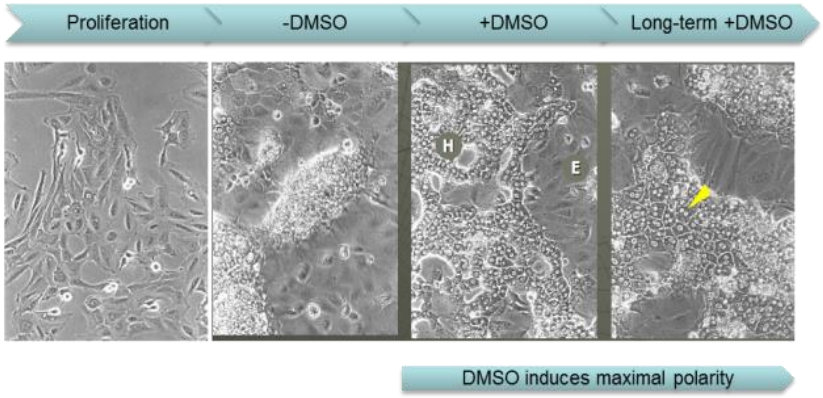
Improved Drug Development Models

- Powerful in vitro tool for predicting drug compound efficacy/ toxicology
- Knockout human POR gene – master controller of drug (CYP450) metabolism
- Can create a suite of context-specific 2D-3D cell lines for Pharma/ Academia
- Delineate CYP450-dependant and –independent effects
  - eg reactive metabolites

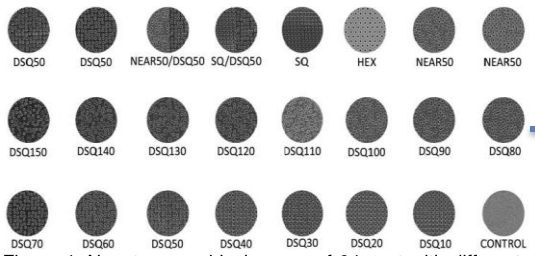
Technology convergence: HepaRG or iPSC-based BioChips

The Future…

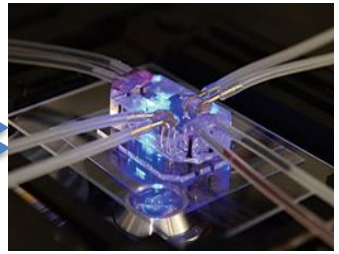
HepaRG progenitor (or iPSC-HLCs) cell line



Impedance biosensor arrays (polymer electrodes/ substrate)



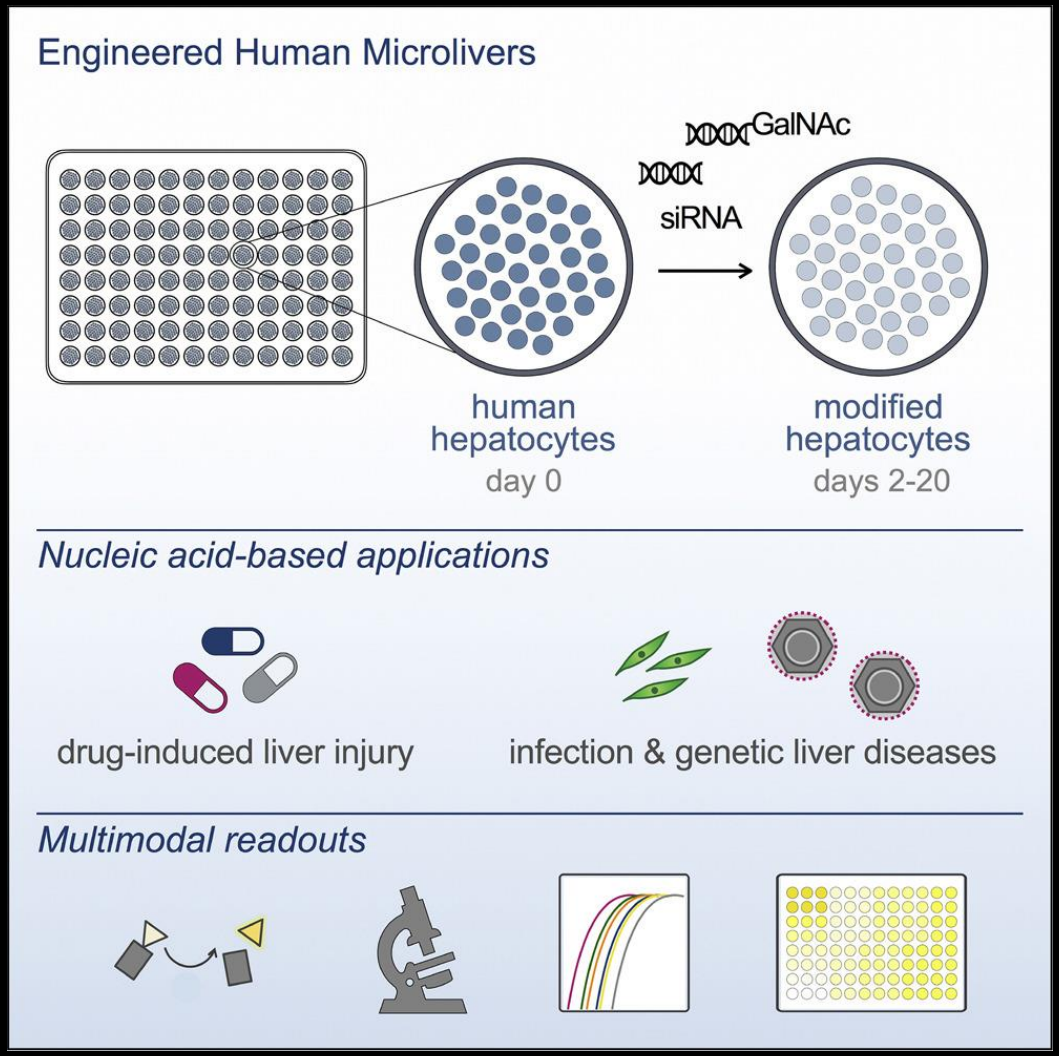
Nano-topographical arrays (University of Glasgow)



Hybrid Biosensor + microfluidics

Readout:
Non-invasive
Cell behaviour

Improving Drug Discovery by Nucleic Acid Delivery in Engineered Human Microlivers



Liliana Mancio-Silva, Heather E. Fleming, Alex B. Miller, Stuart Milstein, Abigail Liebow, Patrick Haslett, Laura Sepp-Lorenzino, Sangeeta N. Bhatia
Cell Metabolism . Volume 29, Issue 3, Pages 727-735.e3 (March 2019) DOI: 10.1016/j.cmet.2019.02.003

Funding

EPSRC
Engineering and Physical Sciences Research Council

BIOPREDIC INTERNATIONAL

HepaRG
The hepatic stem cell line

PRO-EURO DILI NETWORK
Prospective European Drug-induced Liver Injury Network

BBSRC
bioscience for the future

CHIEF SCIENTIST OFFICE

Thank you!

The University of Edinburgh

Leonard J Nelson, PhD
Institute for Bioengineering

PROFILE

Senior Researcher in Synthetic Biological Engineering

COST-DILI MC substitute

RESEARCH

- Human liver modelling
- DILI/ Liver disease models
- Non-invasive screening technologies

WG3

Preclinical *Human* Models

- 2D-3D HepaRG-based organotypic models
- Immunomodulation + Kupffer cells (THP-1)
- Vascular + LSECs (TRP3)
- Innervation + neurons
- Disease + NAFLD
- Advanced Liver BioChips
  - Microfluidics
  - Impedance biosensing

Non-invasive technologies

- Phenotypic profiling
- Label-free | Real-time | HTS
- Optical and chemical imaging platforms:
- 3D-CARS
- 3D-OCT
- ECIS – Impedance biosensing
- Metabolic imaging [O_2]