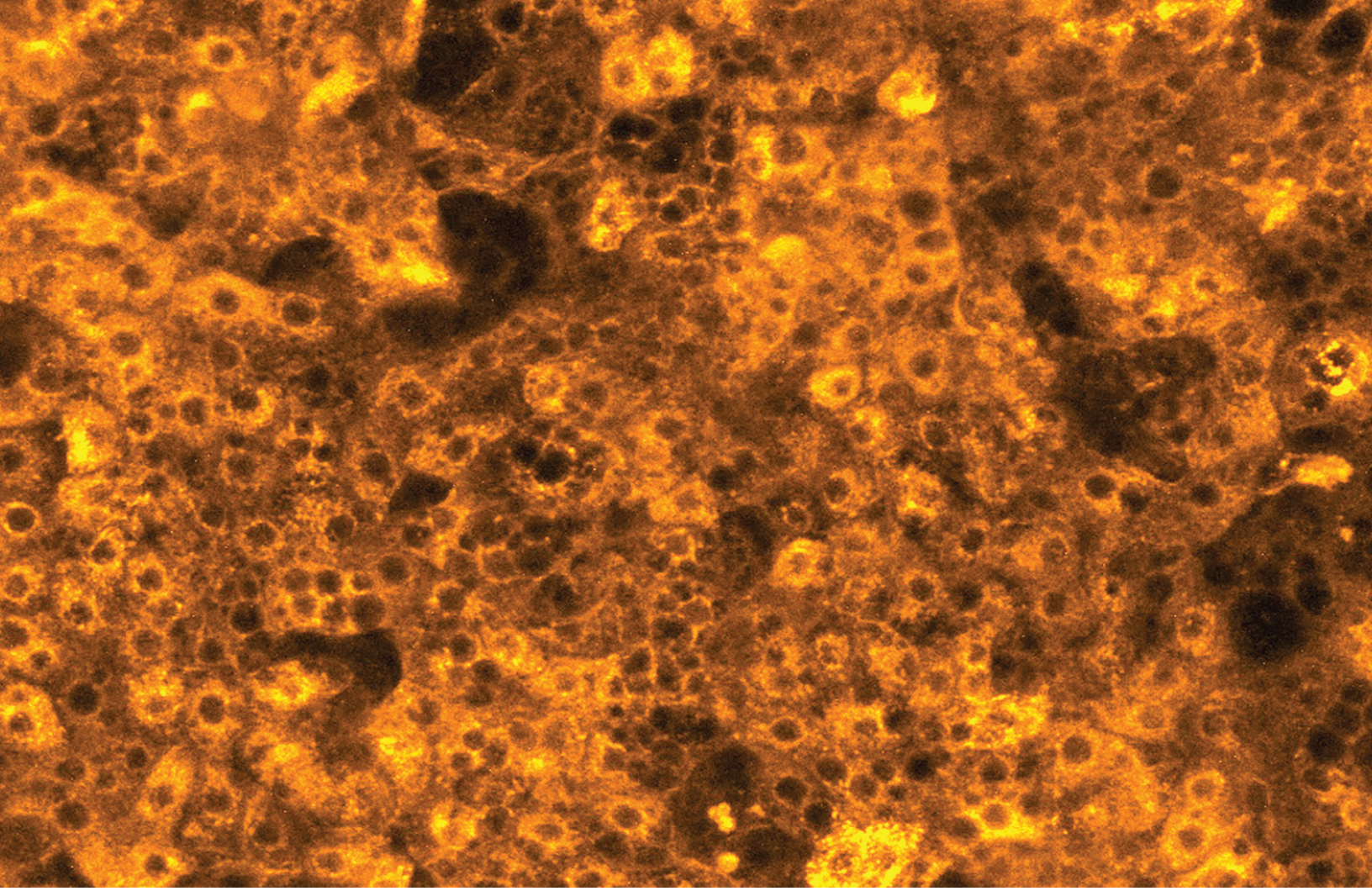


ioHepatocytes

Human iPSC-derived hepatocytes

Powered by opti-ox™

Consistent. Defined. Scalable.

Learn more about ioHepatocytes

ioCells™

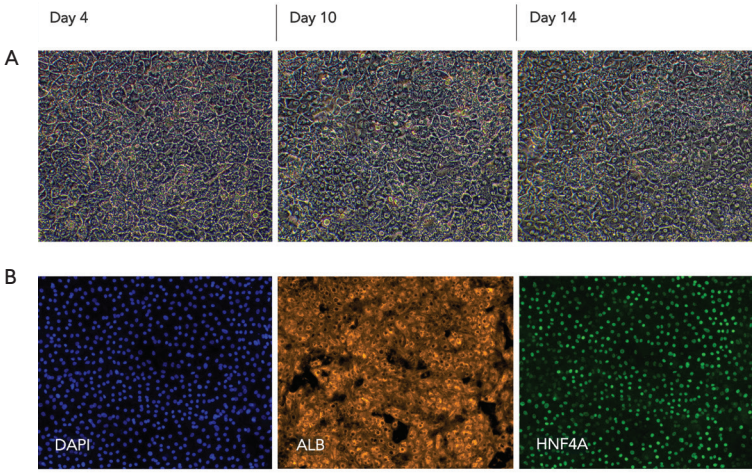
About the cells

ioHepatocytes (Discovery Research) are human iPSC-derived hepatocytes, deterministically programmed using opti-ox* technology. The cells display a cobblestone morphology, prominent nuclei and expression of core hepatocyte markers from day 4.

Highly characterised and defined, so you know exactly what is in every vial

A) Brightfield images of ioHepatocytes (Discovery Research) at day 4, 10 and 14. Images show expected cobblestone morphology and well-defined borders.

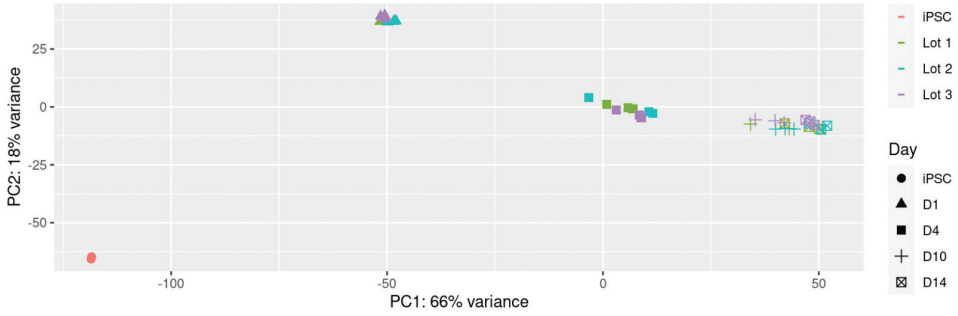
B) Immunofluorescence staining at day 14 showing homogenous expression of pan-hepatocyte markers ALB (orange) and HNF4A (green), and the DAPI counterstain (blue).



Transcriptome analysis reveals high lot-to-lot consistency

Bulk RNA sequencing analysis was performed on three separate lots of ioHepatocytes (Discovery Research). Principal component analysis represents the variance in gene expression between samples.

This analysis shows high consistency between each lot of ioHepatocytes across all timepoints. Day 10 samples cluster closely to Day 14 samples, indicating transcriptomic similarity between these time points. iPSCs controls cluster far from programmed cells from all time points analysed.

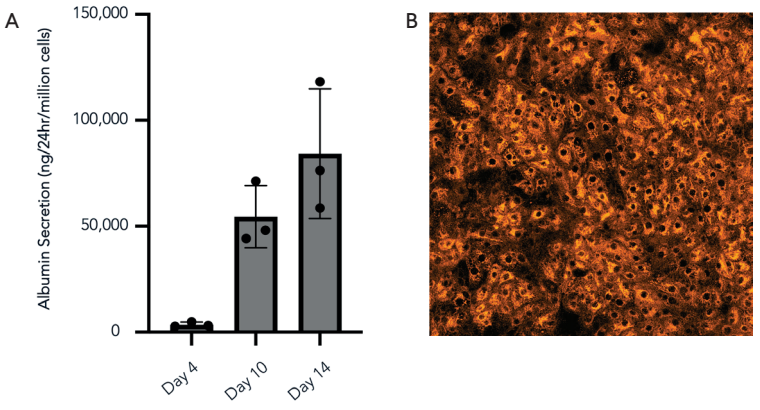


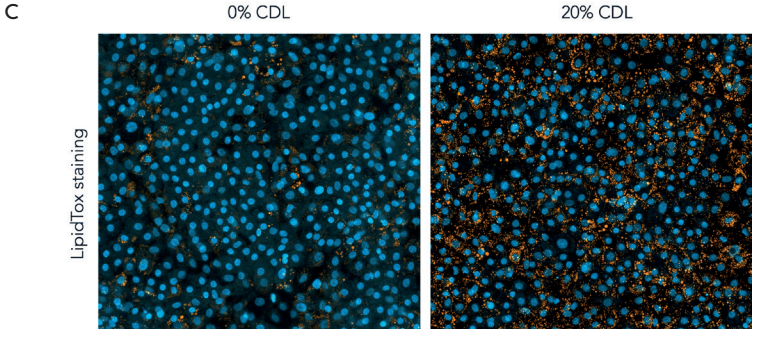
ioHepatocytes secrete albumin and accumulate lipids

A) Albumin secretion over time (ng/24hr per million cells) generated via ELISA at day 4, 10 and 14. At day 4 cells are secreting around 5,000 ng per million cells. As cells continue to mature, so does their metabolic competency. With secretion measured at >50µg to >80µg from day 10 - 14 post-thaw.

B) Albumin expression by immunofluorescence staining at day 14 post-thaw.

C) To explore the use of ioHepatocytes (Discovery Research) to model MASLD we have treated our cells with a 0% and 20% lipid concentrate solution and performed lipid tox staining. Immunofluorescence imaging demonstrates an increase in lipid accumulation (orange) at the elevated concentration. DAPI (blue) was included as a counterstain.





About the cells

ioHepatocytes (Toxicology) are human iPSC-derived hepatocytes, deterministically programmed using opti-ox technology. The cells express phase I-III drug metabolism genes and maintain functional CYP3A, CYP2B6, and CYP1A2 enzymes to support advanced metabolic investigations. ioHepatocytes (Toxicology) also demonstrate DILI responses comparable to primary human hepatocytes, providing a highly predictive platform for hepatotoxicity assessment.

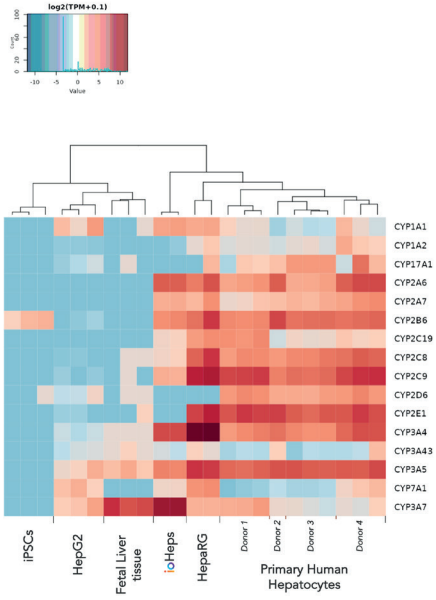
Expression of Phase I, II and III metabolism genes

Expression of genes involved in Phase I – Cytochrome P450 enzymes (left) and Phase II and III (right) drug metabolism. Bulk RNA seq was performed on ioHepatocytes (Toxicology) and on HepaRG after 8 days in culture. Data from Fetal liver, fresh and plated primary hepatocytes (PHH) was sourced externally and used for comparison.

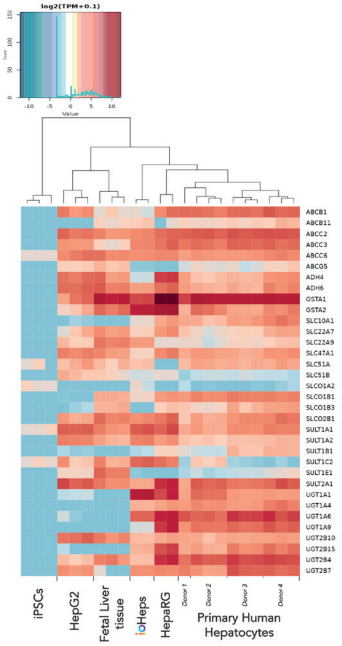
Data shows that ioHepatocytes have a high similarity in expression profile with primary human hepatocytes. Specifically, ioHepatocytes cluster closely with PHH and HepaRG and separately from the more immature HepG2 and fetal liver tissue.

Importantly, ioHepatocytes express the key enzymes CYP3A4, CYP2B6, CYP2C9, GSTA1/2 and UGT1A1, ABCB11 and ABCC2.

Phase I metabolism - CYP450 enzymes



Phase II & III metabolism

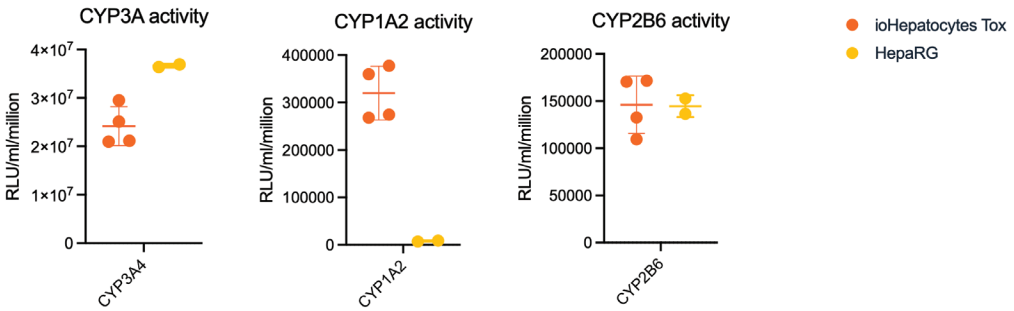


ioHepatocytes can be used to model drug-induced liver toxicity in vitro

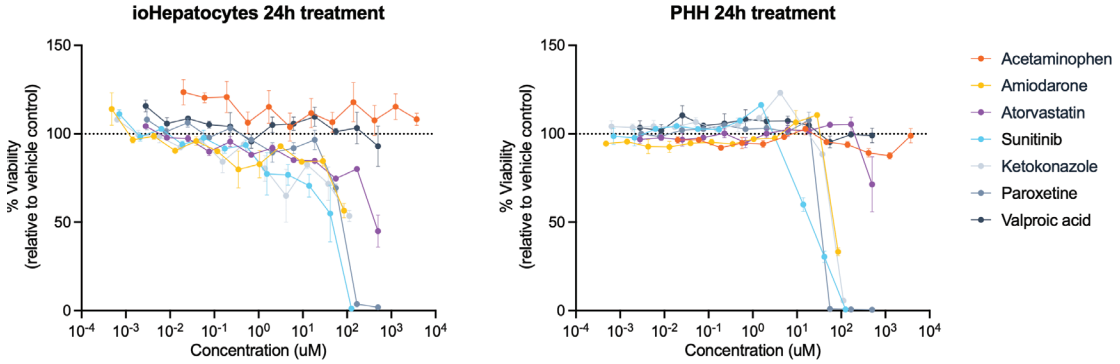
A) Plots showing activity for CYP3A, CYP2B6 and CYP1A2 using the relevant CYP-glo assays from Promega. ioHepatocytes present high functionality for all the CYP enzymes tested and are higher than HepaRGs for CYP1A2.

B) Plots showing viability of ioHepatocytes (Toxicology) and PHHs after 24h exposure to drugs known to cause DILI. Data shows similar cytotoxic response in both cell types except for Atorvastatin, which shows higher cytotoxicity in ioHepatocytes. Please note that Atorvastatin is known to be cytotoxic in 3D models of hepatocytes but not 2D models (*Procter et al. 2017*), suggesting ioHepatocytes are better suited to model cytotoxic response to this drug. Valproic acid was used as a control as known to not affect cell viability in vitro.

A



B



Product information

Cat code

Starting material
Human iPSC line

Seeding compatibility
6, 24 and 96 well plates

Shipping info
Dry ice

Donor
Caucasian adult male (skin fibroblast)

Vial size
Small: >5 x 10^6 viable cells

Quality control
Sterility, protein expression & gene expression

Differentiation method
opti-ox™ deterministic cell programming

Recommended seeding density
>400,000cm^2

User storage
LN2 or -150°C

Format
Cryopreserved cells

Product use
ioCells™ are for research use only

Applications
Metabolic disease modelling
Research and drug discovery
Toxicity testing

Focus Area / Assay	ioHepatocytes Discovery Research	ioHepatocytes Toxicology
CYP450 Induction/Inhibition	P450 Inhibition studies	**Recommended:** P450 Induction/ Inhibition studies
Metabolic clearance	General metabolite screening	**Recommended:** Precise mapping of Phase I, II and III clearance rates
Target ID / validation	Large-scale CRISPR or phenotypic screens	
Albumin & Urea secretion	High-sensitivity functional output / longitudinal monitoring	
Acute cytotoxicity (ATP/LDH)	General compound safety	**Recommended:** Broader Phase I, II and III enzyme expression / activity to capture cytotoxic effects that may not be identified using the Discovery Research product
Idiosyncratic DILI prediction	DILI assessment / predictivity of compounds that are not metabolised by CYP / P450 enzymes	**Recommended:** To detect toxic reactive metabolites by enhanced P450 activity
Mitochondrial toxicity	Assessing respiratory impact and OCR/ECAR changes in metabolically active cells	
MASH/MAFLD phenotyping	Lipid accumulation (steatosis) and inflammatory marker studies	
Viral infection (HBV/HCV)	A stable, reproducible host for viral entry and replication assays	
Fibrosis co-culture	Integration of either product with HSCs to monitor collagen deposition and activation	
Immune-mediated hepatotoxicity	Provision of a suitable system, depending on toxicity pathway that is being investigated	Recommended: Co-culture with Kupffer cells to provide an immune-mediated system for assessing hepatotoxicity

Application suitability

Context-dependent Optimal

Who we are

bit.bio combines the concepts of cell programming and biology to provide human cells for research, drug discovery and cell therapy, enabling a new generation of medicines.

This is possible with our deterministic cell programming technology opti-ox – a gene engineering approach that enables unlimited batches of any human cell to be manufactured consistently at scale.

For general information, email info@bit.bio

To learn more, visit www.bit.bio

BB/HEP/io1028EA23/V1

bit.bio
THE CELL CODING COMPANY