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Poster 237

Development of a Fully Isogenic iPSC-Derived Multicellular Liver Model for Disease Modelling and Toxicity Screening

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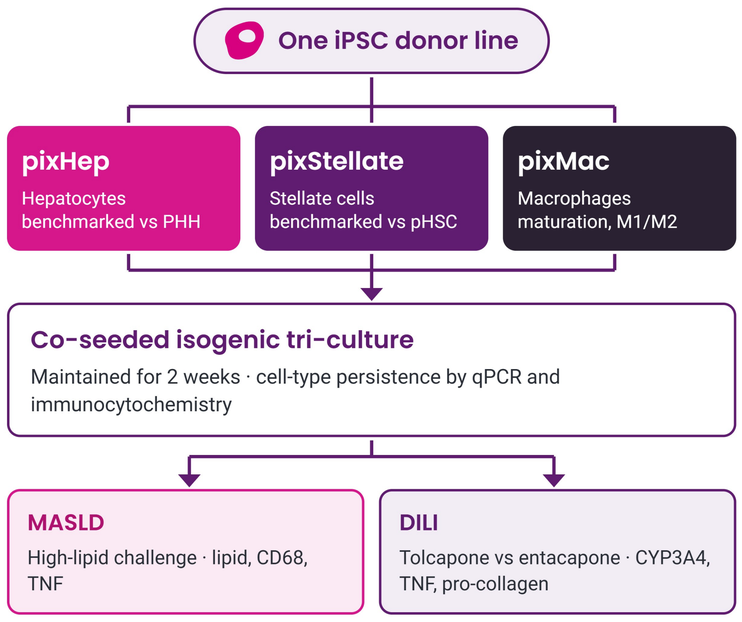
[1] Pixl Bio Ltd, Babraham Research Campus, Cambridge, United Kingdom

Introduction

Physiologically relevant liver models need parenchymal, fibrogenic and immune cell populations working together. Building them from cells of different donors introduces genetic variability that complicates data interpretation.

We developed a **fully genetically matched** multicellular liver model in which iPSC-derived hepatocytes, hepatic stellate cells and macrophages are all generated from the **same donor**.

Study design



Methods

Hepatocytes were benchmarked against primary human hepatocytes (PHH) for hepatic marker expression, albumin secretion, Phase I and Phase II enzyme expression and CYP3A4 activity.

Hepatic stellate cells were compared with primary human HSCs (pHSC) on lineage-marker expression.

Macrophages were assessed for maturation markers and polarisation towards M1 (IFNγ + LPS) and M2 (IL-4) phenotypes.

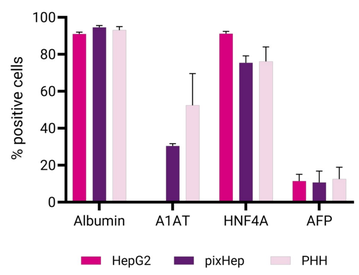
Tri-culture: the three cell types were combined and maintained for two weeks; persistence was evaluated by qPCR and immunocytochemistry.

Challenges: tri-cultures received a high-lipid diet to model MASLD, or tolcapone (hepatotoxic) vs entacapone (non-hepatotoxic) to model DILI.

1 pixHep resemble primary human hepatocytes

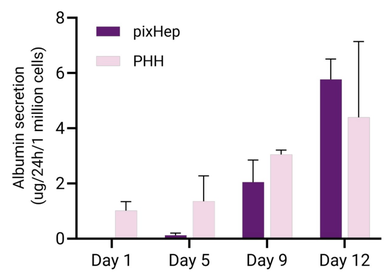
Hepatic identity, albumin output and drug-metabolism capacity comparable to PHH; HepG2 shown for reference

A Maturity markers



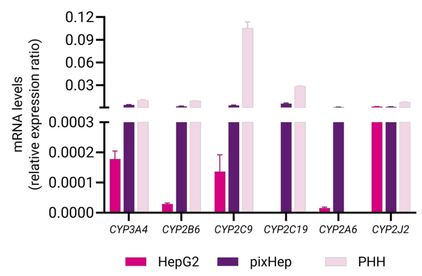
% positive cells: albumin, A1AT, HNF4A, AFP

B Albumin secretion



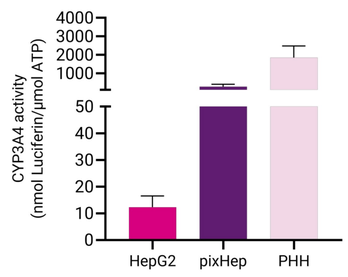
Albumin secretion, days 1–12, pixHep vs PHH

C Phase I – CYP450 genes



CYP3A4, 2B6, 2C9, 2C19, 2A6 and 2J2 mRNA

D Basal CYP3A4 activity

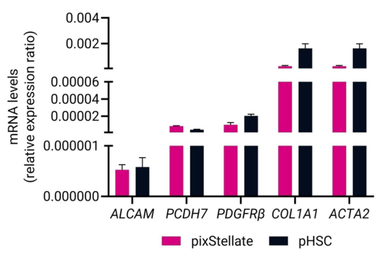


Luminescent CYP3A4 activity, normalised to ATP

2 pixStellate match primary HSCs

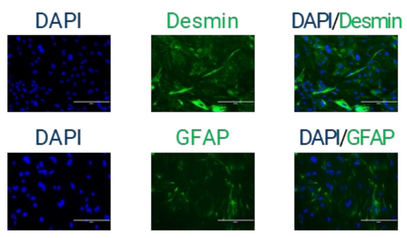
Lineage markers on par with primary HSCs

A HSC marker expression (qPCR)



ALCAM, PCDH7, PDGFRβ, COL1A1, ACTA2 mRNA vs pHSC.

B Immunofluorescence



Desmin and GFAP (green), DAPI nuclei (blue).

Stellate identity confirmed by Desmin/GFAP staining and HSC-marker expression (PDGFRβ, COL1A1, ACTA2), comparable to primary HSCs.

3 pixMac are mature, tunable macrophages

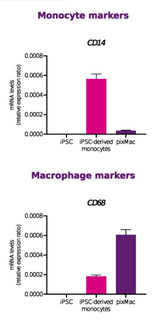
Myeloid identity, maturation and M1/M2 polarisation

A Morphology



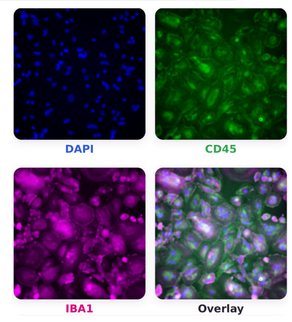
M0 macrophages, phase contrast, 10×.

B qPCR



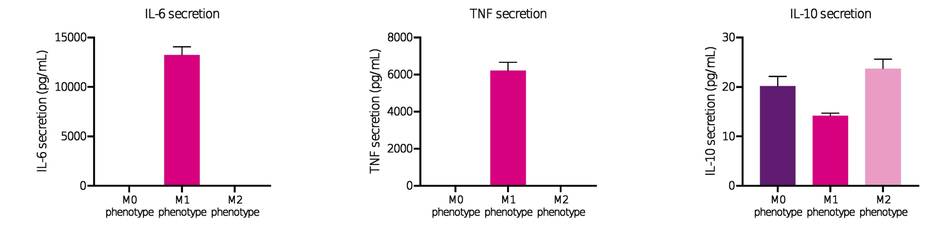
CD14 lost, CD68 gained.

C Immunofluorescence



CD45 and IBA1 co-expression.

D Functional polarisation: cytokine secretion



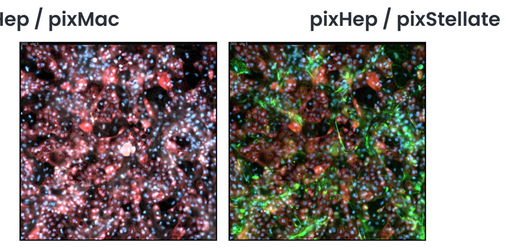
M0 polarised for 24 h with IFNγ + LPS (M1) or IL-4 (M2); secreted cytokines by ELISA.

CD45⁺ CD68⁺ IBA1⁺ macrophages. M1 secretes high IL-6 and TNF; M2 restores IL-10 secretion.

4 A stable isogenic tri-culture

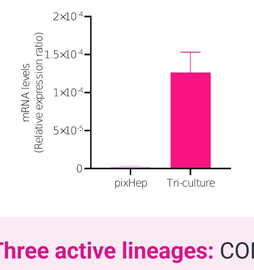
All three lineages persist for at least two weeks

A Immunocytochemistry

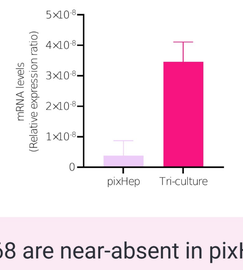


Albumin hepatocyte · **GFAP** stellate · **IBA1** macrophage

B Stellate marker



C Macrophage marker

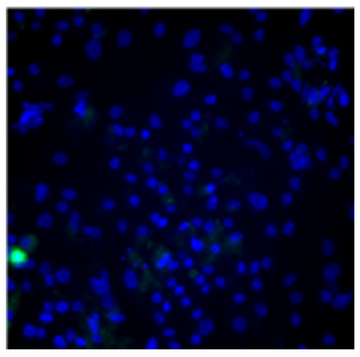


Three active lineages: COL1A1 and CD68 are near-absent in pixHep monoculture and rise sharply in tri-culture.

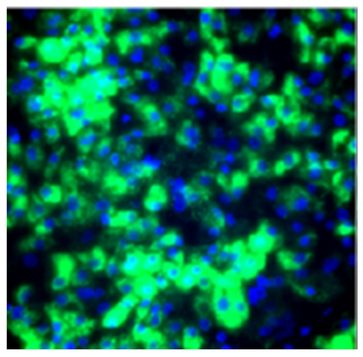
5 Modelling MASLD

High-lipid challenge drives steatosis and inflammation

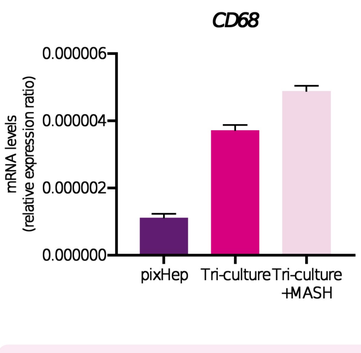
A Control diet



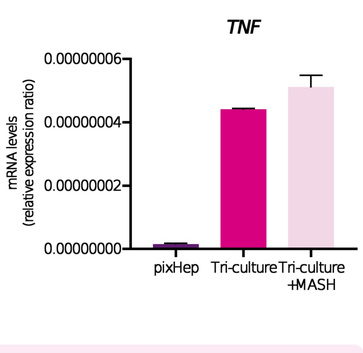
B High-lipid



C CD68 mRNA



D TNF mRNA

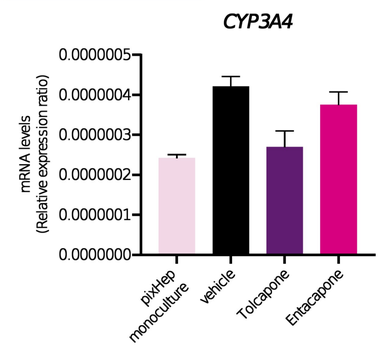


MASLD features recapitulated. At 2 weeks, high-lipid (MASH diet) exposure increases lipid accumulation (BODIPY green, DAPI blue) and upregulates CD68 and TNF.

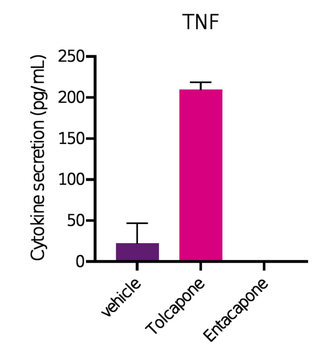
6 Tri-culture flags DILI

Tolcapone (hepatotoxic) vs entacapone (non-hepatotoxic)

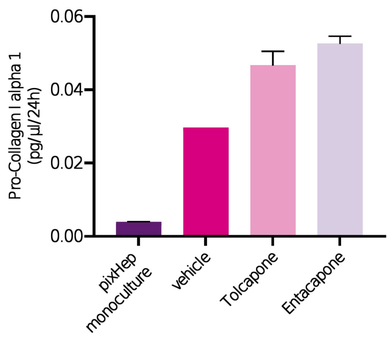
A CYP3A4 mRNA



B Secreted TNF



C Pro-collagen I α1



Why this pair

Two COMT inhibitors from the same class: **tolcapone** causes human liver injury, **entacapone** does not.

Tolcapone's liability was missed by conventional preclinical testing, making the pair a benchmark for human-relevant DILI models.

Immune readout flags DILI: tolcapone separates cleanly on secreted TNF and suppresses CYP3A4; entacapone does not.

CONCLUSIONS

**One donor.
Three lineages.
One well.**

01 **Genetically controlled.** All three lineages from one iPSC donor remove donor-to-donor variability from the readout.

02 **Benchmarked and stable.** pixHep and pixStellate resemble their primary counterparts, pixMac polarise to M1 and M2, and all three persist together for at least two weeks.

03 **Safety- and disease-relevant.** Immune-mediated DILI is flagged by secreted TNF, and a high-lipid challenge recapitulates MASLD.