Evaluating liver microfluidic system models for a Next Generation Risk Assessment

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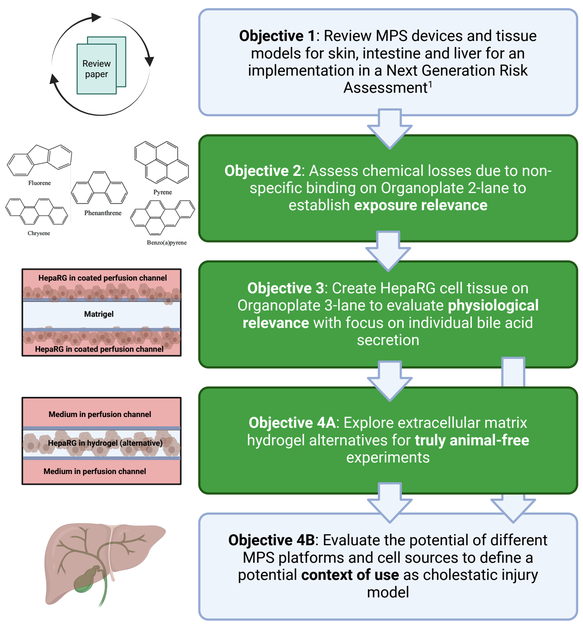
GOAL

Several commercial and academic microfluidic system (MPS) devices claim to recapitulate *vivo like* functions from cells, yet only a few independent studies have evaluated Liver MPS performances and their potential value in a Next Generation Risk Assessment toolbox. This project provides key insights from an end user perspective in toxicological research, focussing on:

- **Exposure relevance**: model and measure chemical distribution and non-specific binding of compounds to *in vitro* compartments
- **Physiological relevance**: metabolic competency and synthetic functioning, with emphasis on individual bile acid secretion
- **Defining a potential context of use**: cholestatic injury model using benchmark chemicals
- **Truly animal-free approach**: Reduce animal-derived cell culture materials

WORKFLOW



Objectives highlighted in green are featured on the poster.

CONCLUSIONS

Objective 2:

- The adjustable mass balance model allows insights into bound and freely available chemical fractions.
- Adaptions to other cell system require verification measurements (e.g. ECM proteins, diffusion, plastic type).

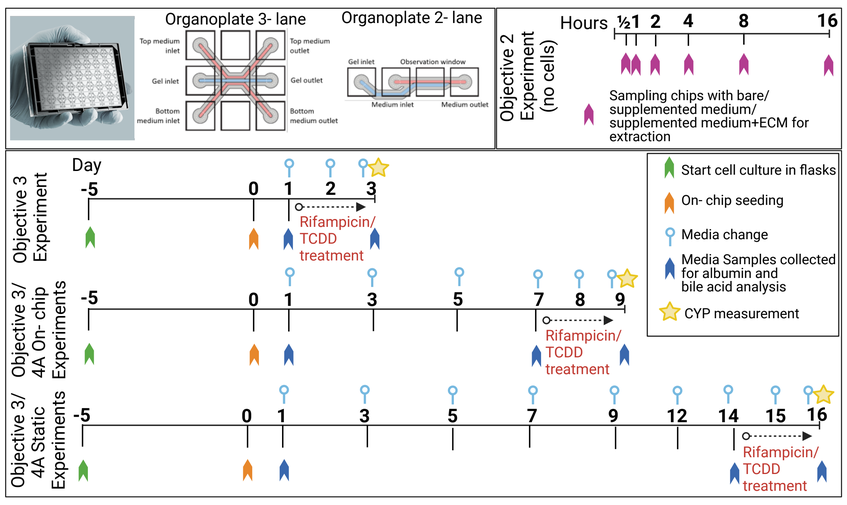
Objective 3:

- Undifferentiated HepaRG cells, seeded on the Organoplate 3-lane, matured without DMSO supplementation within the first days, drastically shortening existing 14 days protocols.
- Synthetic functioning needs improvement[3].
- CYP induction response was observed, showing metabolic competence.
- Glycine- and tauro conjugated bile acids were *de novo* synthesised, yielding in a human-comparable bile profile, given that 30% of the total are tauro- conjugates[4].
- Cells responded to Rifampicin and TCDD by reducing bile acid secretion, proposing potential use as cholestatic injury model.

Objective 4A:

- Animal-free ECM hydrogels provide viable alternatives based on application needs, with PeptiMatrix 7.5 emerging as a promising on-chip candidate. However, further optimization of culture conditions are required.

MPS DEVICE AND EXPERIMENTAL TIMELINES



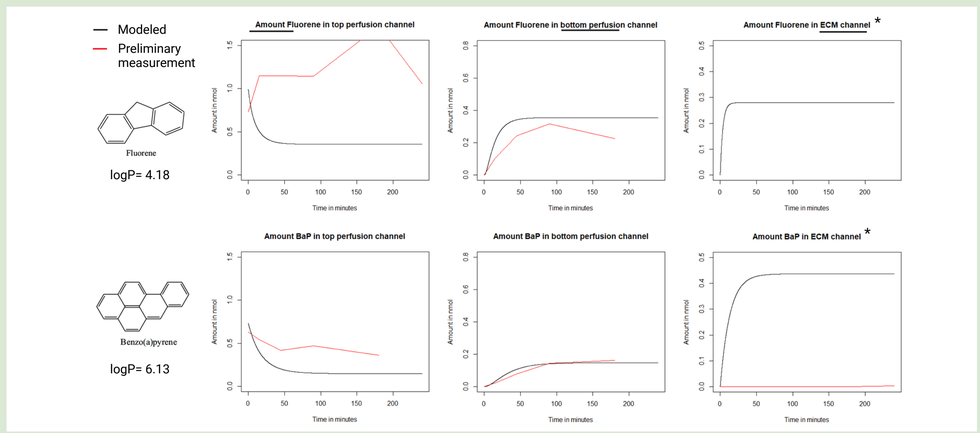
Organoplate 3-lane and 2-lane with layouts and experimental timelines.

Objective 2 experiments used six sampling time points to determine bound and free chemical amounts

Objective 3 experiments matured HepaRGs on-chip and evaluated physiological relevance at three independent time points: 3, 9 or 16 days.

Objective 4A experiments matured HepaRGs in hydrogel suspension over 9 days dynamically or over 16 days in static condition with increasing DMSO supplementation

RESULTS



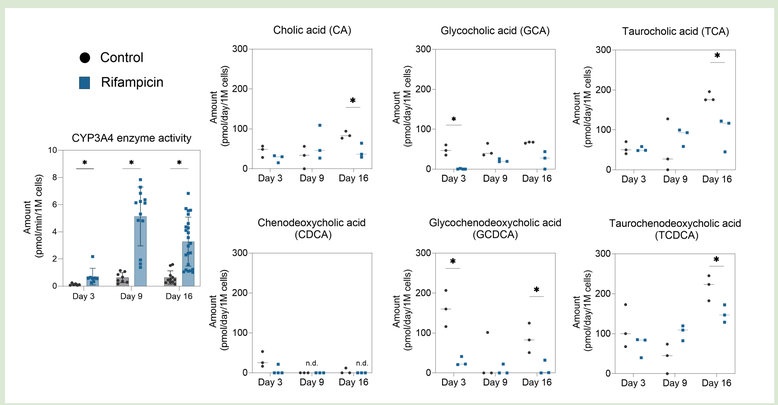
* Chemicals could not be recovered from proteinous ECM matrix in this preliminary experiment.

Mass balance model delivers adjustable framework to assess binding

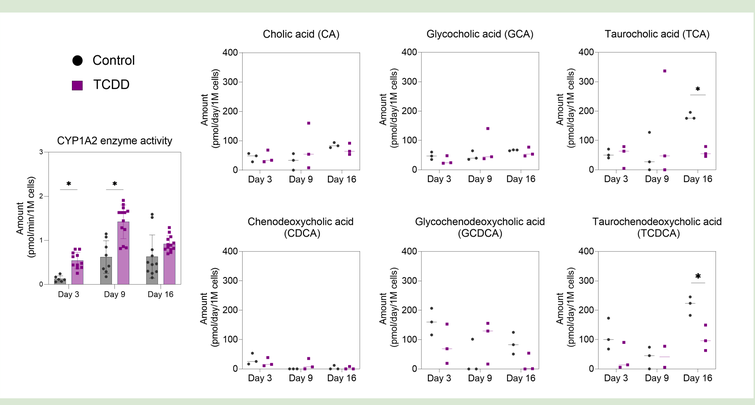
Objective 2 Differential distribution of bound and unbound Fluorene and B[a]P amounts, showing findings of mass balance model and preliminary measurement (GCMS). Results consider partitioning to serum, plastic, ECM proteins and HepaRG cell lipids in Organoplate 3-lane.

After preliminary measurements, a refined protocol with improved recovery of lipophilic chemicals from plastic and proteinous matrices was developed for the Organoplate 2-lane to extract bound samples for GC-MS analysis (data analysis is ongoing and manuscript with detailed protocols in preparation).

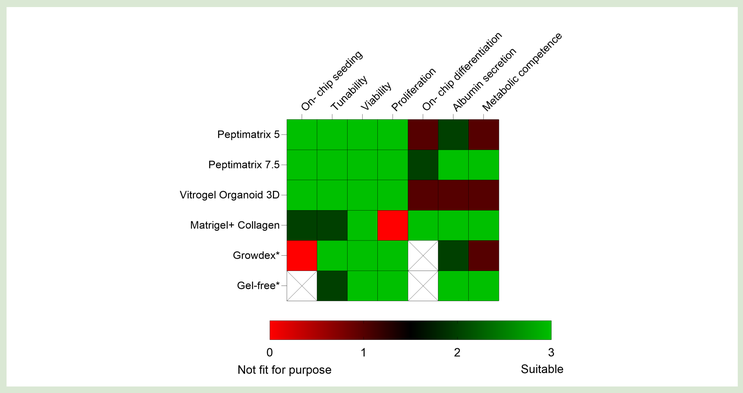
CYP induction treatments show metabolic competence



Strongest rifampicin and TCDD treatment effects on bile acid secretion occurred predominantly after 16 days



Objective 3 Assess the metabolic competency and synthetic functioning of HepaRGs cells on the 3-lane Organoplate at three independent culture time points, undifferentiated HepaRG were seeded in the perfusion channels against Matrigel and matured under flow without DMSO supplementation. CYP1A2 and CYP3A4 enzyme induction treatment was started on either day 1, 7 or 14 with TCDD (10 nM) or rifampicin (25 µM) exposure over 48 hours, respectively. Basal and induced enzyme activity was measured with CYP Glo assay (Promega). Individual bile acids were measured with LCMS method published by de Bruijn et al (2022)[2] and normalised with bile acid amounts found in serum supplemented medium.



All tested animal- free ECM hydrogels supported cell proliferation but optimisation for on-chip differentiation is needed

Objective 4A Five hydrogels were tested in *static and dynamic (Organoplate 3- lane) condition for their biocompatibility to create a viable and metabolic competent HepaRG culture. Cells were matured in hydrogel suspension by supplementing with DMSO in static (over 14 days) or introducing flow in dynamic condition (over 7 days). Viability and proliferation was measured with WST-8 assay, albumin secretion with ELISA and CYP 3A4 enzyme activity was assessed after 48 hour rifampicin induction (25 µM) with CYPGlo assay (Promega).

OUTLOOK

Liver-MPS demonstrates strong potential as a cholestatic injury model, leading to a research collaboration with the Rusyn Lab at Texas A&M to explore **Objective 4B** using benchmark chemicals. Additionally, the mass balance model from **Objective 2** will assess the exposure relevance of the benchmark compounds.

ACKNOWLEDGEMENT

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REFERENCES