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A liver- on- chip to evaluate bile acid secretion for the use in a Next- Generation Risk Assessment

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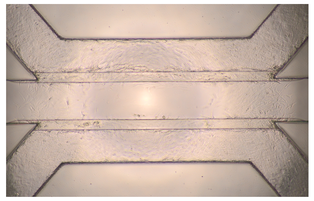
Liver- on- chip

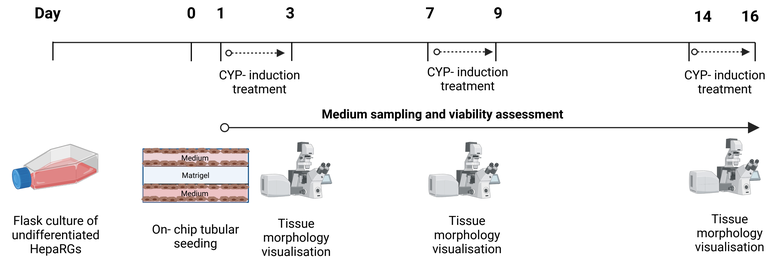


Bright- field image of the liver tissue on the 3- lane Organoplate (Mimetas B.V.)

Liver models are required to **evaluate for chemical biomodulation and biotransformation**, as well as for **mechanism- based hepatoxicity** studies[1]. Within a Next-Generation Risk Assessment toolbox, Organ- on- chip systems offer the potential to generate data which can be used in a higher tier approach for **biokinetic refinements, targeted biological mechanism testing and point of departure estimation**[2].

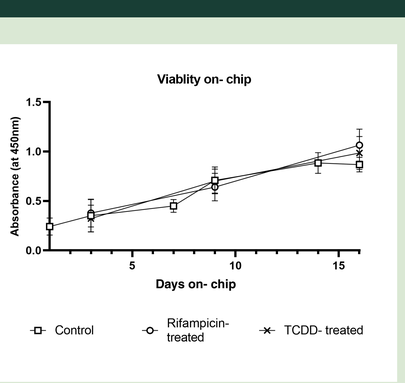
This study aimed to assess **liver- relevant functional baseline markers** for this **medium- throughput system** at different time points after a **DMSO- free differentiation on- chip** to evaluate the **suitability as a cholestasis model.**

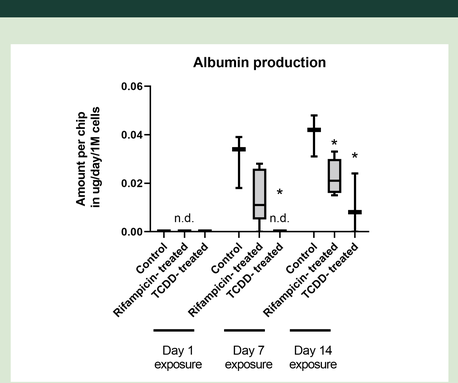
Differentiation and treatment on- chip

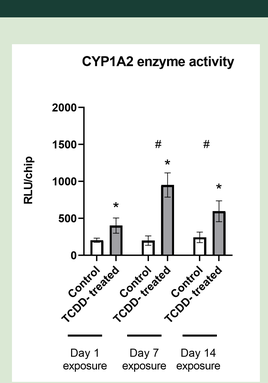


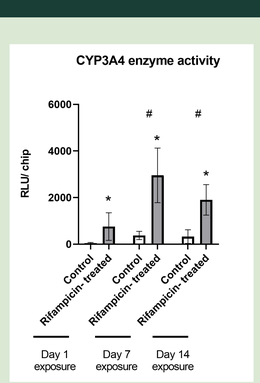
Overview of differentiation and treatment on- chip. HepaRG cells (Biopredic) were precultured in- flask and seeded against Matrigel in the medium channels of the 3-lane Organoplate (Mimetas B.V.). The liver-tissue was treated for 48h for CYP enzyme induction with Rifampicin or TCDD **on either Day 1, Day 7 or Day 14**. For all cultures, the tissue morphology was visualised, the medium sampled and the viability assessed on Day 1, as well as on the exposure start and end days to determine secretion profiles and cytotoxicity.

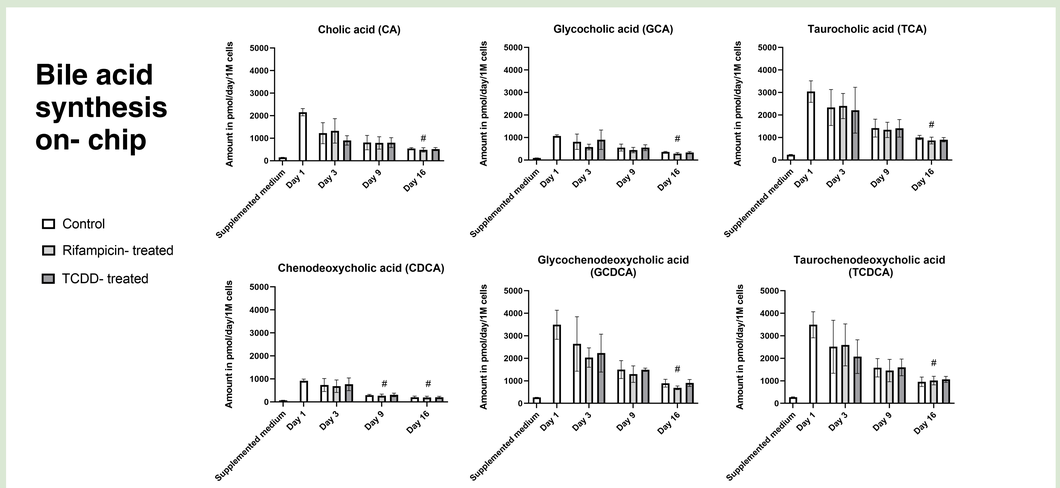
Functional baseline

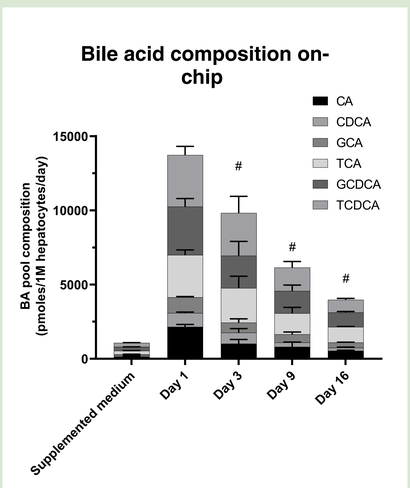












Functional measurements cell viability (WST- 8 assay), albumin production (ELISA), CYP enzyme activity (Promega P450 Glo) and bile acid synthesis (LCMS) of the HepaRG liver on- chip to evaluate the model robustness at different time points (n=3). * n.d. = not detected (< LOD); Values represent the mean ±SD of triplicate measurements of at least three independent experiments.

Results summary

- HepaRGs remained viable on- chip but **entered the Matrigel at early time points** (data not shown).
- The tissue differentiated without dimethyl sulfoxide (DMSO) within the first day on-chip (see CYP induction)
- Albumin production increased over the duration of culture. However, the tissue produces only a **fraction of albumin** compared to other reported perfused liver- models, suggesting a **too high shear stress** for this seeding set-up.
- **Under treatment with TCDD and Rifampicin** (hepatotoxins known to alter protein biosynthesis), the **albumin production declined as expected**.
- **Metabolic competency** for CYP1A2 and 3A4 was the highest for induction treatment after 7 days on- chip.
- A substantial amount of glycine- and tauro conjugated bile acids was *de novo* synthesised, resulting in a **human-comparable liver and bile profile**, given that 30% are tauro- conjugates. The perfused model produced also **more bile acids compared to reported static models**[3], even though levels decreased over time.
- De novo bile acid synthesis decreased with the duration of culture and **did not demonstrate a treatment effect**
- The bile acid pool decreased overall but remained at the **same composition ratios**

Collectively, the data demonstrates that HepaRGs on- chip produce an *in vivo* like bile acid profile but also that more culture set- up refinement is needed to increase the functional baseline as a fit- for- purpose cholestasis model in a Next- Generation Risk Assessment toolbox.

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References