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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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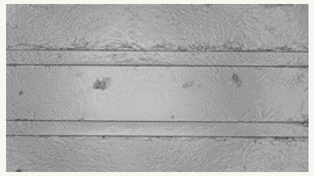
Liver- on- chip

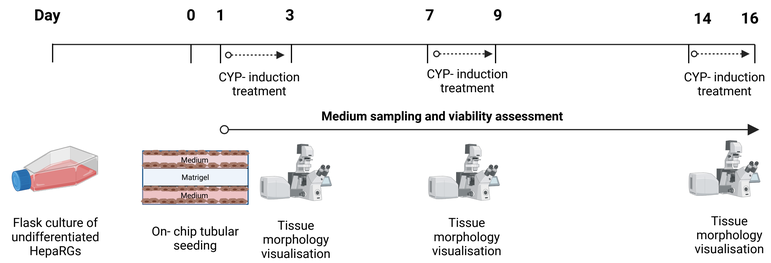


Bright- field image of the liver tissue after 7 days on the 3-lane Organoplate (Mimetas B.V.)

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].

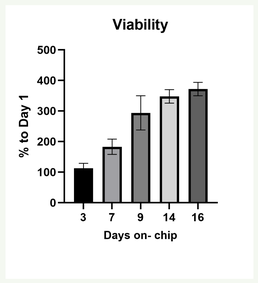
This study aimed to develop a **self-organizing** HepaRG-liver model on the Organoplate, with the objective to analyse **morphology and functional baseline** at three independent time points to evaluate the suitability as a **cholestasis model**.

Differentiation and treatment on- chip

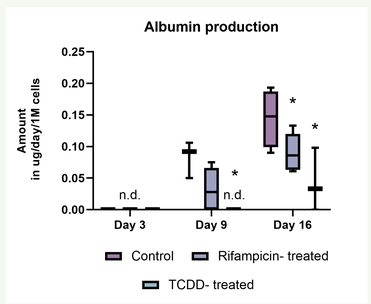


Overview of differentiation and treatment on- chip. HepaRG cells (Biopredic) were precultured in- flask and seeded against Matrigel in the medium channels of the 3-lane Organoplate (Mimetas B.V.). The cells were treated for 48h with Rifampicin or TCDD to induce CYP enzyme activity **on either Day 1, 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.

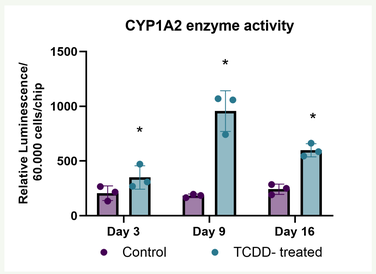
Functional baseline



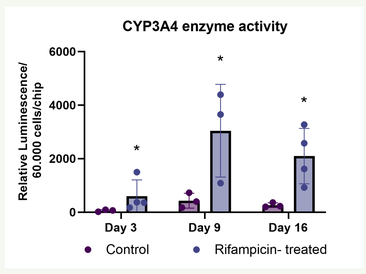
Viability of untreated cells relative to Day 1 on- chip. Measured with WST-8. Data graphed as mean ±SD (n=3)



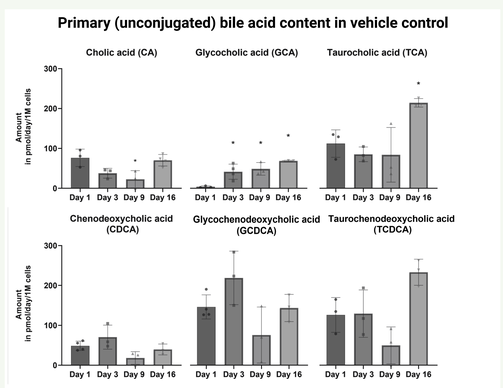
Time- course investigation of albumin. Measured with ELISA. n.d.= not detected. *p < 0.05 by one way ANOVA followed by Dunnetts multiple comparison test. Data graphed as mean ±SD (n=3)



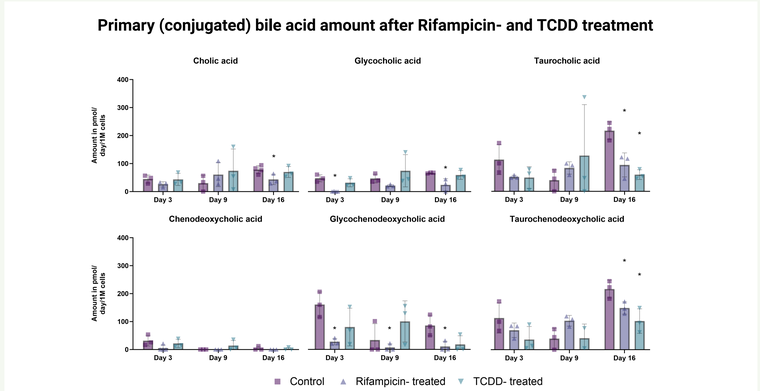
CYP1A2 enzyme activity after 48 hour TCDD-treatment. Measured with P450 Glo (Promega). *p < 0.05 by unpaired t- test with Welchs correction. Data displayed as mean ±SD (n=3)



CYP3A4 enzyme activity after 48 hour Rifampicin- treatment. Measured with P450 Glo (Promega). *p < 0.05 by unpaired t- test with Welchs correction. Data displayed as mean ±SD (n=3)



Time- course investigation of individual primary (conjugated) bile acid profile in medium. Measured with LCMS. *p < 0.05 by one way ANOVA followed by Dunnetts multiple comparison test. Data displayed as mean ±SD (n=3)



Time- course investigation of individual primary (conjugated) bile acid profile in TCDD- and Rifampicin treated chip. Measured with LCMS. *p < 0.05 by unpaired t- test with Welchs correction. Data displayed as mean ±SD (n=3)

Results summary

- HepaRGs remained viable on- chip but **entered the Matrigel at early time points**.
- The tissue differentiated without dimethyl sulfoxide (DMSO) within the first day on-chip (see CYP induction)
- Albumin production gradually 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[3].
- Lower albumin production under treatment with TCDD and Rifampicin**.** TCDD is a known protein biosynthesis inhibitor, the mechanism of Rifampicin remains unclear[4].
- **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 bile profile**, given that 30% are tauro- conjugates[5].
- Most sensitive period for changes in the bile acid profile after treatment occurred predominantly after day 14, especially for Rifampicin and to a lesser extent for TCDD

Our findings indicate rapid maturation and early metabolic and synthetic capabilities of HepaRGs- on- chip within just three days. While albumin synthesis fell short of expectations, the bile acid profile mirrored human *in vivo* conditions. Notably, this is the first study to explore individual bile acid profiles in a liver-on-chip model, revealing significant reductions in secretion similar to human protective responses after Rifampicin treatment. Thus, the HepaRG-liver-on-chip may provide a powerful *in vitro* Next- Generation Risk assessment tool for investigating dysregulated mechanisms in bile acid homeostasis.

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References