A MICROFLUIDIC THYROID-LIVER PLATFORM TO INVESTIGATE THYROID TOXICITY MECHANISMS IN HUMANS AND RATS

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 # 2789

OBJECTIVE

The assessment of human relevance of thyroid toxicity observed in animal assays still represents a major challenge. Distinguishing between species-specific aspects of the mechanism of thyroid disruption is also a recurring problem. In addition, the delineation between direct effects on the thyroid gland function and indirect or secondary responses on the hypothalamus-pituitary-thyroid function, e.g. increased hepatic clearance of thyroid hormones, has major impact on the safety risk assessment of chemicals.

Rodents, which are widely used for regulatory toxicity testing, are particularly sensitive to perturbations of the thyroid homeostasis. Although thyroid hormone production and function is similar in humans and rodents there are some important species differences in the transport, metabolism and catabolism of thyroid hormones. However, there is currently no distinctive assay available to test and evaluate direct vs. indirect thyroid hormone dysregulations and the relevance to humans.

Here we present ongoing work towards the development and characterization of a human and rat liver-thyroid *in vitro* system. A commercially available multi-organ-chip platform enables co-cultivation and thereby organ crosstalk of three-dimensional (3D) thyroid and liver organ models.

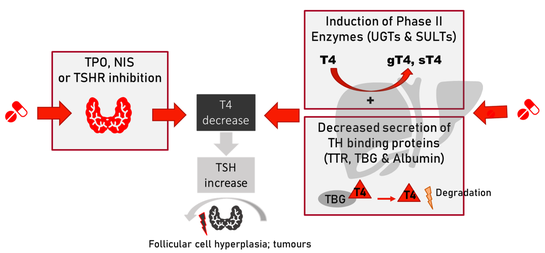
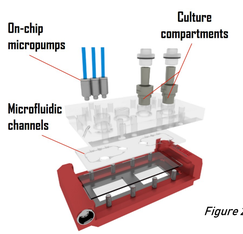


Figure 1: Potential direct and indirect thyroid toxicities for which prediction is aimed to be simultaneously covered within one in vitro assay.

MULTI-ORGAN CHIP SYSTEM



The dynamic system allows tissue-to-tissue communication as the culture compartments are directly connected via microfluidic channels. An on-chip micropump enables a near to physiological fluid-to-tissue ratio as well as a long-term cultivation of cells and 3D organoids.

HUMIMIC Chip2

Figure 2: Desgin of the commercially available HUMIMIC Chip2.

RESULTS

3D LIVER MODEL

MRP2 EXPRESSION AND ALBUMIN SECRETION

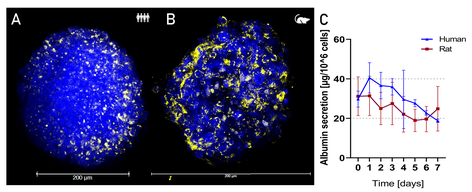


Figure 3: Visualized canaliculi structures and albumin secretion
Morphology of human (day 7) and rat (day 14) liver spheroids were assessed. Human [A] and rat [B] liver spheroids consisted of 10.000 and 5.000 cells per spheroids, respectively. They were cultured, fixed, stained for cell nuclei (blue) and the bile canalicular transporter MRP2 (yellow) and visualized with confocal microscopy. Scale bars indicate 200 µm. Furthermore, hepatocyte specific [C] albumin production [Mean + SD] was monitored for both species.

PHASE I ENZYME INDUCTION

	Substance	Conc.	Cytochrom P450 Activity [FC to control] Human	Rat
CYP1A2	ß-Naphthoflavone	5 µM	1.6 ± 0.02	21.0 ± 4.18
CYP2B6	Phenobarbitone	1 mM	5.8 ± 0.38	3.5 ± 0.59
CYP3A4	Rifampicin	5 µM	5.5 ± 0.30	1.9 ± 0.87
	Fipronil sulfone	2.5 µM	7.0 ± 1.99	2.8 ± 1.05

Figure 4: Activity of phase I enzymes and its inducibility by known inducers
Human and rat liver spheroids were daily exposed to ß-naphthoflavone, phenobarbitone, rifampicin or fipronil sulfone for seven days. CYP1A2 enzyme activity for ß-naphthoflavone, CYP2B6 for phenobarbitone and CYP3A4 for rifampicin and fipronil sulfone enzyme induction was assessed by P450-Glo™CYP1A2, CYP2B6 and P450-Glo™CYP3A4 AssayKit, respectively.

METABOLISM OF THYROID HORMONES

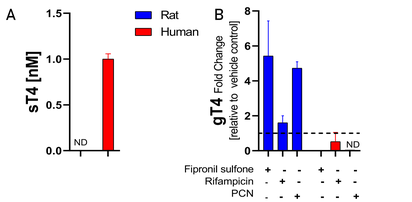


Figure 5: Catabolism of thyroxin by human and rat liver spheroids
[A] Liver spheroids were cultured for 7 days in HUMIMIC Chip2. Thyroxin (T4) was added for 24h and supernatants were analyzed for basal sulfated thyroxine (sT4) levels by LC-MS/MS analysis. B] Liver spheroids were daily exposed to fipronil sulfone (2.5 µM), rifampicin (5 µM) or pregnolone-16-a-carbonitirle (PCN: 5 µM) for 7 days. Thyroxin (T4) was added for 24h and supernatants were analyzed for glucuronidated thyroxine (gT4) by LC-MS/MS analysis. Data is presented as fold changes relative to the vehicle control. [Mean + SEM]. In rats sulfation of thyroxin was not detected (ND), whereas glucuronidation predominates in rat induced liver spheroids.

3D THYROID MODEL

MAINTENANCE OF POLARIZATION

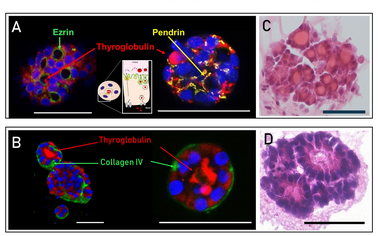


Figure 6: Follicles remain colloidal structure
Thyroid follicles were fixed, stained for thyroid polarization markers and visualized via confocal microscopy after >14 days of culture. [A] Rat follicles remained colloidal structure with cell nuclei (blue), ezrin (green) at the apical domain or pendrin (apical iodine transporter, yellow) and thyroglobulin (red) for. [B] Human follicles remained thyroglobulin (red) and Collagen IV (green) as basal marker. [C,D] H&E staining of rat and human culture respectively confirmed colloidal structure. Scale bars represent 50 µm.

FUNCTIONAL TSH RECEPTOR SIGNALLING

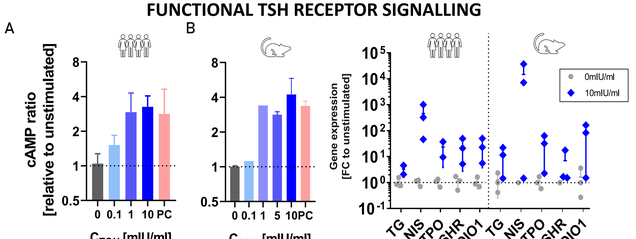


Figure 7: *Thyrotropin (TSH) activates TSH receptor and increases intracellular cAMP concentrations*
[A] Human and [B] rat thyroid follicles were exposed to TSH or a positive control (PC,10µM forskolin) Thereafter cAMP levels were measured. For human as well as rat thyroid follicles TSH induced intracellular cAMP signaling, a critical mediator of thyroid hormonogenesis.

Figure 8: TSH induces expression of genes relevant for thyroid hormone biosynthesis
Gene expression profiling of human and rat thyroid follicles (fold change relative to non stimulated follicles). Expression of hormone biosynthesis relevant genes such as thyroglobulin (TG), sodium symporter (NIS), thyreoperoxidase (TPO), thyroid stimulating hormone receptor (TSHR) and deiodinase (DIO1) are induced after stimulation with 10 mIU/mL TSH

THYROID HORMONE SECRETION

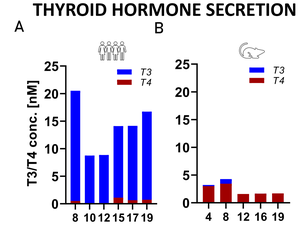


Figure 9: Thyroid hormone secretion remains stable for both species over > 2 weeks
[A] Human and [B] rat thyroid follicles were cultured for 19 days and stimulated every 2-4 days with 5 and 10 mIU TSH, respectivley.
[Supernatants were analyzed for thyroxin (T4) and triiodothyronine (T3) by LC-MS/MS analysis.

COCULTURE OF LIVER & THYROID

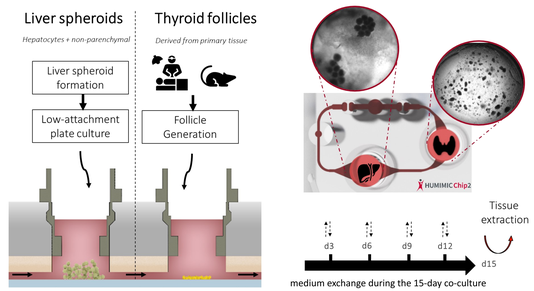


Figure 10: Generation and set-up of a 2-week coculture of liver and thyroid organ equivalents

FUNCTIONALLY ACTIVE FOR 15 DAYS

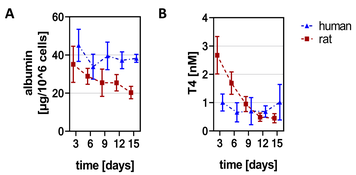


Figure 11: Liver and thyroid organoids maintain albumin secretion and thyroxin (T4) synthesis over 15 days in co-culture.
[A]Albumin concentrations were measured by photorimetric via an antigen-antibody reaction.
[B] T4 levels in supernatant were assessed by LC-MS/MS analysis.

HEPATIC GENE EXPRESSION PROFILE

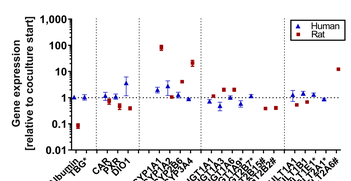


Figure 12: Overall gene expression of co-cultured liver spheroids remains stable.
Hepatocyte specific and thyroid hormone relevant markers as well as phase I and II enzymes were analyzed. Rat] Fold changes of 15-day co-cultured liver spheroids were calculated relative to freshly isolated spheroid cell mixture. Human] Fold changes of 15-day co-cultured liver organoids were calculated to reference liverspheroids at the start of co-culture. (* = human specific; # = rat specific)

SUMMARY & OUTLOOK

For the first time, a human and rat liver-thyroid *in vitro* system was established. Liver as well as thyroid organoids feature vital properties and functions.

3D liver organoids respond to reference inducers by increased cytochrome P450 activity. Futhermore human and rat liver spheroids maintain the ability to eliminate the thyroid hormone thyroxine (T4). Inducible T4 glucuronidation is the predominat elemination pathway in rat liver spheroids, whereas elemination via T4 sulfation occures in human but not rat liver spheroids. This data therefore nicely reflect a characteristic difference in T4 catabolism between the two species in vivo .

The in vitro cultured 3D thyroid follicles retain a colloidal structure, show an inducible TSH receptor signaling and secrete T4 and T3. Thus, our model is a closer representation of the thyroid functional unit *in vivo* than conventional 2D thyroid models.

Liver and thyroid organoids can be viably co-cultured within the HUMIMIC Chip2 over a time course of at least 15 days.

Thus, our *in vitro* assay presents a promising platform to simultaneously determine direct as well as indirect effects within one single assay. This assay will represent a major step forward to outline species similarities/differences with a significant contribution to the 3R principles. Future planned in depth model characterization and system qualifications will demonstrate the reliability of the liver thyroid test platform.

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