

# 3D organotypic liver cultures using the hanging drop method for *in vitro* toxicity studies

Daniel Mueller<sup>1</sup>, Anika Koetemann<sup>1</sup>, Valery Shevchenko<sup>2</sup>, Christophe Chesné<sup>2</sup>, Christiane Guguen-Guillouzo<sup>2</sup>, Elmar Heinzle<sup>1</sup> and Fozia Noor<sup>1</sup>

<sup>1</sup>Biochemical Engineering Institute, Saarland University, Saarbruecken, Germany

<sup>2</sup>Biopredic International, Rennes, France

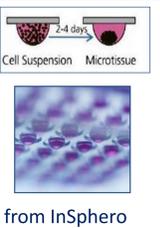
## Abstract

The major aim of the NOTOX consortium is to develop and validate predictive mathematical and bioinformatic models characterizing long-term toxicity responses. Thereby, organotypic human cell cultures will be developed for long-term repeated-dose toxicity testing. We cultivated HepG2 and HepaRG cells in 3D organotypic cultures using the hanging drop method (InSphero, Zurich, Switzerland). 250–8000 seeded HepG2 cells formed spheroids within 2–3 days which increased in size during the first week. H&E (hematoxylin and eosin) staining of HepG2 spheroid slices indicates disc-like structures of the organotypic cultures and significant improvement in single cell morphology compared to 2D cultures. Liver specific albumin production was higher in the HepG2 organotypic cultures as compared to both monolayer and collagen-sandwich cultures. CYP1A induction capacity was significantly improved by organotypic cultivation. The acute toxicity (24 h) of tamoxifen, an anti-cancer drug, was lower in the 3D cultures as compared to monolayer and collagen-sandwich cultures, which could be explained by a higher drug efflux through membrane transporter (MRP-2). Organotypic cultivation of differentiated HepaRG cells was carried out using the same method. 500–8000 seeded HepaRG cells formed compact spheroids within 3-4 days which did not increase in size since these cells do not further proliferate under used conditions.

We conclude that the 3D organotypic cultivations can be readily performed and can be used for the investigation of long-term repeated-dose toxicity and metabolism. Additionally, they show potential in high-throughput screening of test compounds and long-term toxicity studies

## Material and methods

The spheroids of HepG2 and HepaRG were produced using the GravityPlus system (InSphero, Zurich, Switzerland; see picture). Albumin was quantified using an inverse ELISA-Kit (Exocell). CYP1A activity in HepG2 cells was induced by incubating the cells with 3-methylcholantrene for 72 h. Activity was then measured by EROD assay. A fluorescence based assay was used for the investigation of MRP-2 transporter activity. The membrane permeable and non-fluorescent substrate 5-chloromethylfluorescein diacetate (CMFDA) is converted by cellular esterases to a membrane-impermeable compound, which then reacts with cellular glutathione to glutathione-methylfluorescein (GSMF). GSMF is a substrate of MRP-2 and is excreted out of the cell into canaliculi.



## Results

### HepG2 and HepaRG spheroids

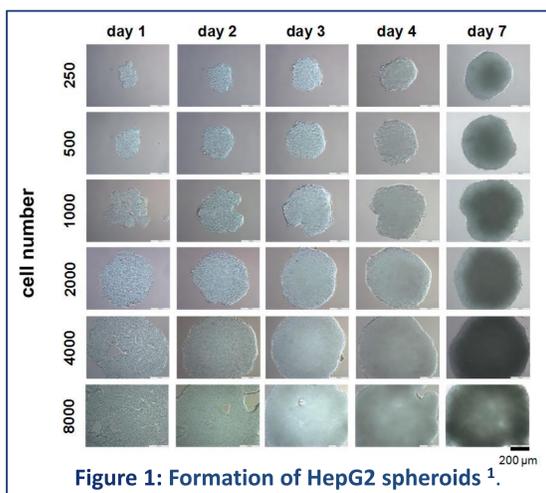


Figure 1: Formation of HepG2 spheroids<sup>1</sup>.

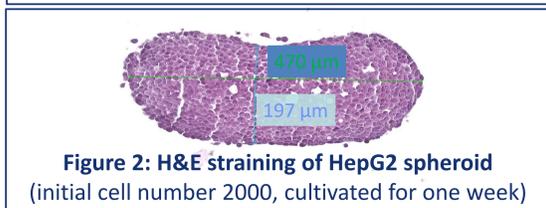


Figure 2: H&E staining of HepG2 spheroid (initial cell number 2000, cultivated for one week)

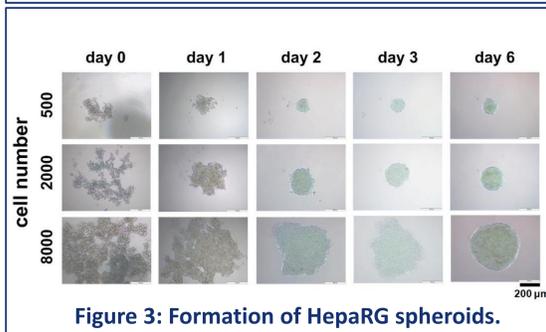


Figure 3: Formation of HepaRG spheroids.

### Increased albumin production and CYP1A induction of HepG2 spheroids

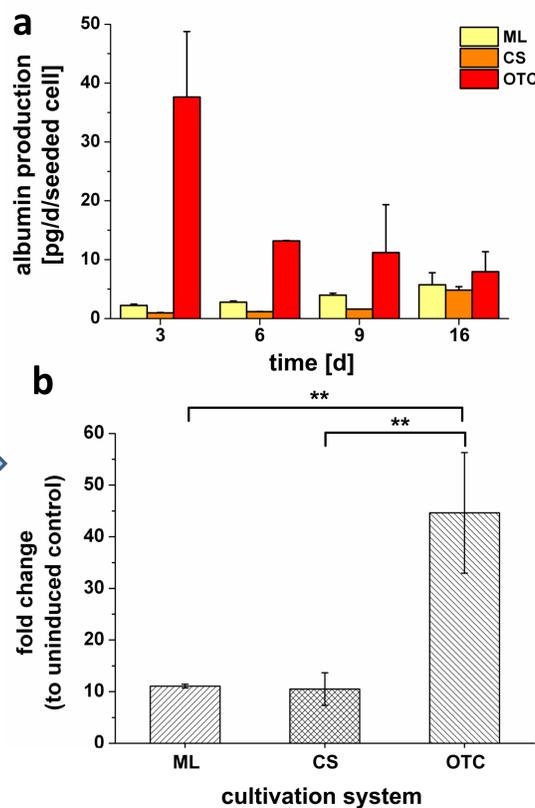


Figure 4: a) albumin production in HepG2 cells maintained in monolayer (ML), collagen sandwich (CS) or organotypic cultures (OTC)<sup>1</sup>, b) CYP1A induction assessed by EROD assay. Induction fold change is shown for HepG2 cells cultivated in ML, CS or OTC<sup>1</sup>. \*\* Significance at  $p < 0.01$

### HepG2 spheroids: Lower sensitivity to tamoxifen

Table 1: Acute toxicity (24h) of tamoxifen in HepG2 cells. EC<sub>50</sub> values of monolayer (ML), collagen sandwich (CS) and organotypic cultures (OTC) are presented<sup>1</sup> (n=3).

	ML	CS	OTC
EC <sub>50</sub> [μM]	14 ± 1.4	19 ± 1.0	57 ± 5.4

### Higher MRP-2 activity in HepG2 spheroids

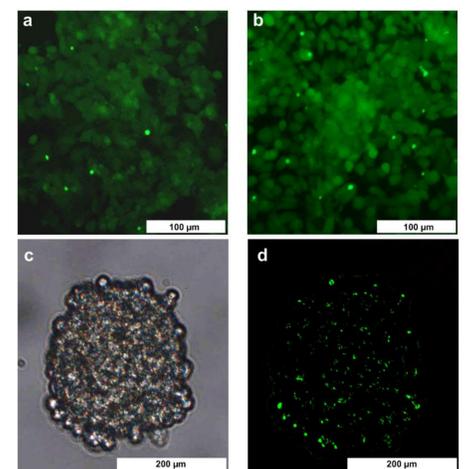


Figure 5: CMFDA-based fluorescence assay for MRP-2 transporter activity in HepG2 a) ML, b) CS, c) OTC (light microscopy) d) OTC<sup>1</sup>.

## Summary

- HepG2 and HepaRG cells form spheroids in hanging drop cultures with adjustable initial cell numbers within 2 – 4 days.
- HepG2 spheroids show increased albumin production and CYP1A induction compared to conventional cultures.
- Lower sensitivity towards acute exposure to tamoxifen was found for HepG2 spheroids, explainable by enhanced drug efflux *via* MRP-2 transporter

<sup>1</sup>Mueller D. Koetemann A. and Noor F. (2011)

J Bioeng Biomed Sci, DOI: 10.4172/2155-9538.S2-002

### Contact:

Daniel Mueller  
daniel.mueller@mx.uni-saarland.de

### Acknowledgements

We thank Esther Hoffmann for technical assistance and InSphero for support.

