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Predicting long-term toxic effects using computer models based on systems characterization of organotypic cultures

3D Spheroid cultivation system for liver cells for drug safety testing

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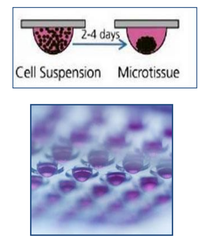
Abstract

We show 3D cultivation of hepatic cell line and primary liver cells as spheroids using the hanging drop method (InSphero Gravity^{Plus} system). Viability and general metabolic parameters of organotypic cultures were analyzed during serum-free maintenance. For the HepG2 organotypic cultures, liver specific albumin production was significantly increased compared to both monolayer and collagen-sandwich cultures. CYP1A induction capacity was improved by the organotypic cultivation. The acute toxicity (24 h) of tamoxifen, an anti-cancer drug, was lower in the 3D HepG2 cultures as again compared to monolayer and collagen-sandwich cultures, which can be explained by a higher drug efflux through membrane transporter which was also proved by the investigation of MRP2-mediated efflux. This indicates that the engineered organotypic cultures could be used for the investigation of CYP450 induction, anti-cancer drug effects and moreover for the study of chemotherapy resistance mechanisms. Moreover, this high-throughput *in vitro* technology is suitable for drug screenings using cell lines as well as primary cells and could serve as alternative to *in vivo* animal testing.

Material and methods

The spheroids of HepG2 and primary human hepatocytes (PHH) were produced using the Gravity^{Plus} system (InSphero AG, Zurich, Switzerland; see picture). Amino acids in the supernatants were quantified by an high performance liquid chromatography (HPLC) method. 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.



from Insphero AG

Results

HepG2 spheroids

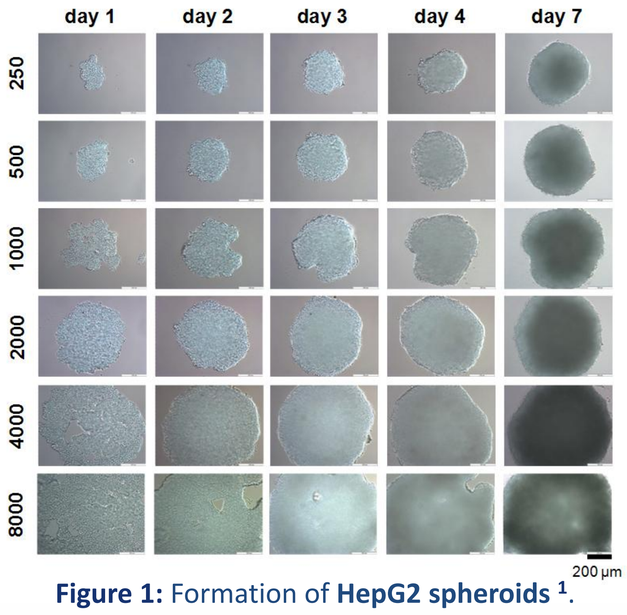


Figure 1: Formation of **HepG2 spheroids** [1].

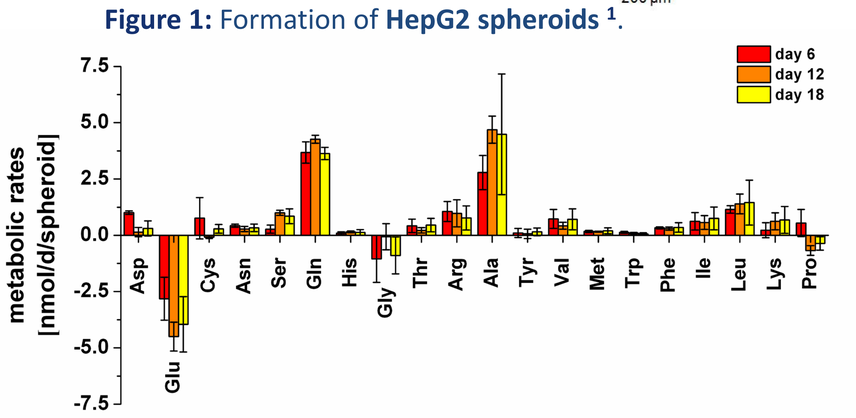


Figure 2: Amino acid metabolism in 3D HepG2 spheroids [1] (day 1 = 1st day in serum-free conditions). Positive vales indicate net uptake.

PHH spheroids

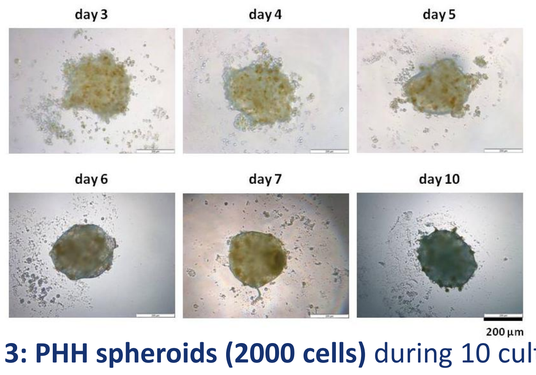


Figure 3: PHH spheroids (2000 cells) during 10 cultivation days.

Increased albumin production and CYP1A induction of HepG2 spheroids

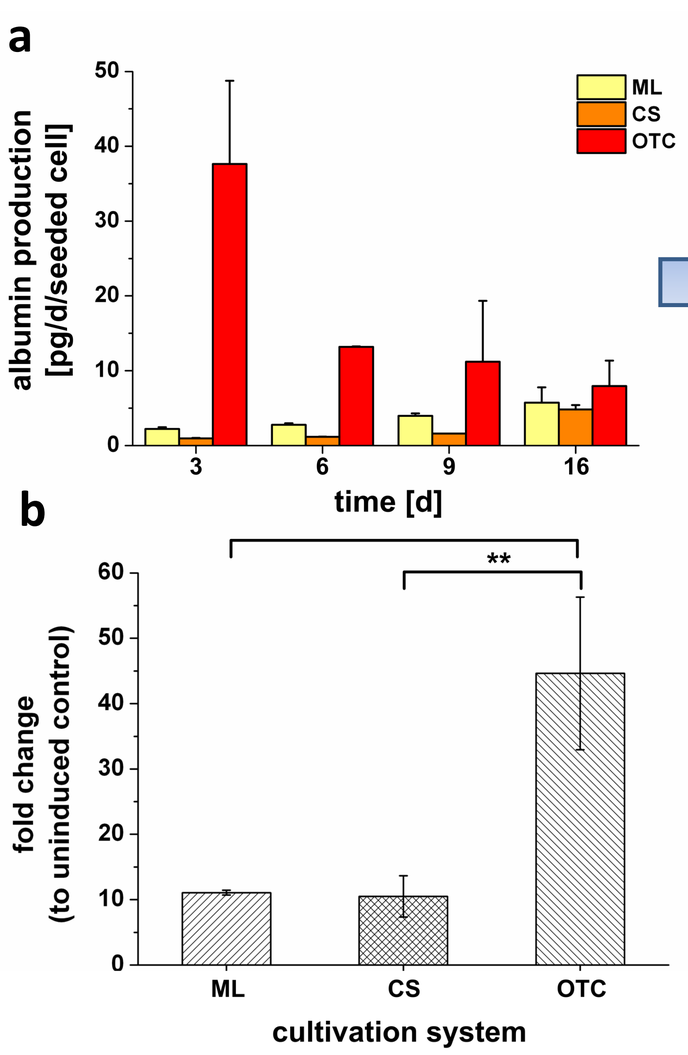


Figure 4: a) albumin production in HepG2 cells maintained in monolayer (ML), collagen sandwich (CS) or organotypic cultures (OTC) [1], **b) CYP1A induction** assessed by EROD assay. Induction fold change is shown for HepG2 cells cultivated in ML, CS or OTC [1]. ** Significance at $p < 0.01$

[1]Mueller D. Koetemann A. and Noor F. (2011)
J Bioeng Biomed Sci, DOI: 10.4172/2155-9538.S2-002

HepG2 spheroids: Lower sensitivity to tamoxifen

Table 1: Acute toxicity (24h) of tamoxifen in HepG2 cells. EC_{50} values of monolayer (ML), collagen sandwich (CS) and organotypic cultures (OTC) are presented[1] *(n=3)*.

	ML	CS	OTC
EC_{50} [µM]	14 ±1.4	19 ±1.0	57 ±5.4

Higher MRP-2 activity in HepG2 spheroids

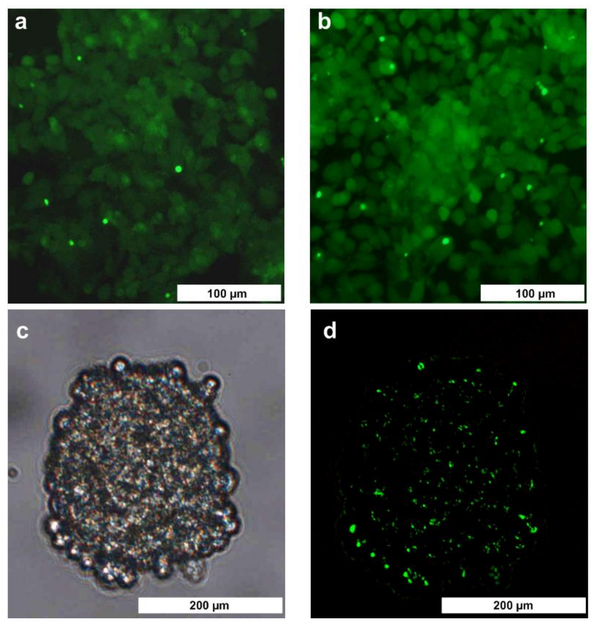


Figure 5: CMFDA-based fluorescence assay for **MRP-2 transporter activity in HepG2**
a) ML, b) CS, c) OTC (light microscopy) d) OTC[1].

Summary

- HepG2 cells and PHH form spheroids in hanging drop cultures with adjustable initial cell numbers within 2 – 4 days.
- HepG2 spheroids show stable amino acid metabolism, as well as 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

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