

3D organotypic cultures of human HepaRG cells: a tool for *in vitro* toxicity studies

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Abstract

The major aim of the NOTOX consortium is to develop and validate predictive bioinformatic models characterizing long-term toxicity responses. Thereby, organotypic human cell cultures will be developed for toxicity testing. In this study, long term 3D organotypic cultures of the human hepatoma HepaRG cell line were prepared, maintained for 3 weeks and assessed for (1) liver specific functions, including phase I enzyme and transporter activities, (2) expression of liver-specific proteins and (3) responses to the hepatotoxic drug acetaminophen. The immunohistochemistry analyses illustrate that these cultures express liver-specific proteins (e.g. albumin, CYP3A4, CYP2E1 and MRP-2) throughout the whole cultivation period. CYP2E1 activity was significantly higher in the 3D versus the 2D cultures. Toxicity studies show that the 3D organotypic cultures are more sensitive to acetaminophen-induced toxicity. Furthermore, the EC₅₀ value (2.7 mM) for acetaminophen on the 3D cultures was similar to *in vivo* toxicity. In summary, these results suggest that the 3D organotypic HepaRG cultures are a promising *in vitro* tool for more accurate assessment of acute and also possibly for chronic drug induced hepatotoxicity.

Material and Methods

The human **HepaRG® cells** (Biopredic International, France) were maintained according to the supplier's recommendations. **3D HepaRG organotypic cultures** were produced using the GravityPLUS™ plates (InSphero AG, Zurich, Switzerland). The **CYP2E1 activity** was assessed by measuring the conversion of chlorzoxazone to OH-chlorzoxazone. **MRP-2 transporter activity** in 2D and 3D HepaRG cultures was measured using the fluorescence-based CMFDA-assay, whereby a substrate of the membrane transporter MRP-2 is formed and excreted out of the cell into the bile canaliculi. For **immunohistochemistry**, HepaRG spheroid cultures were fixed and collected on culture days 10 and 21 and sent to Histo-Center AB for further processing and IHC analyses of CYP2E1, MRP2, CYP3A4, albumin and Ki67. For **acute toxicity studies**, 2D and 3D HepaRG cultures were exposed to acetaminophen (0.5 - 80 mmol/L) for 24 hours on culture days 4 and 21. Cell viability was assessed using ATP assay kit (Promega, Sweden) according to the manufacturer's recommendations.

Results

3D HepaRG spheroid formation

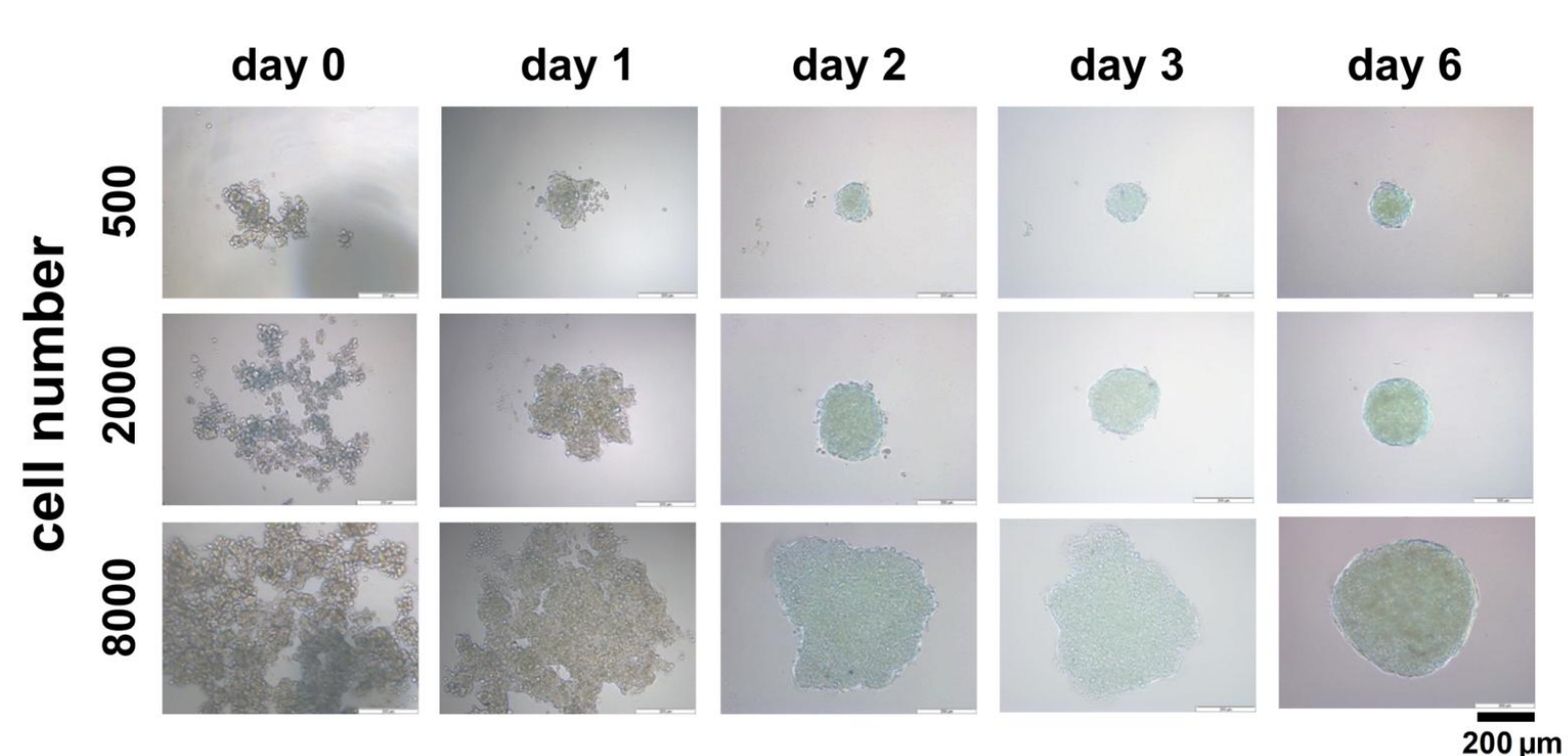
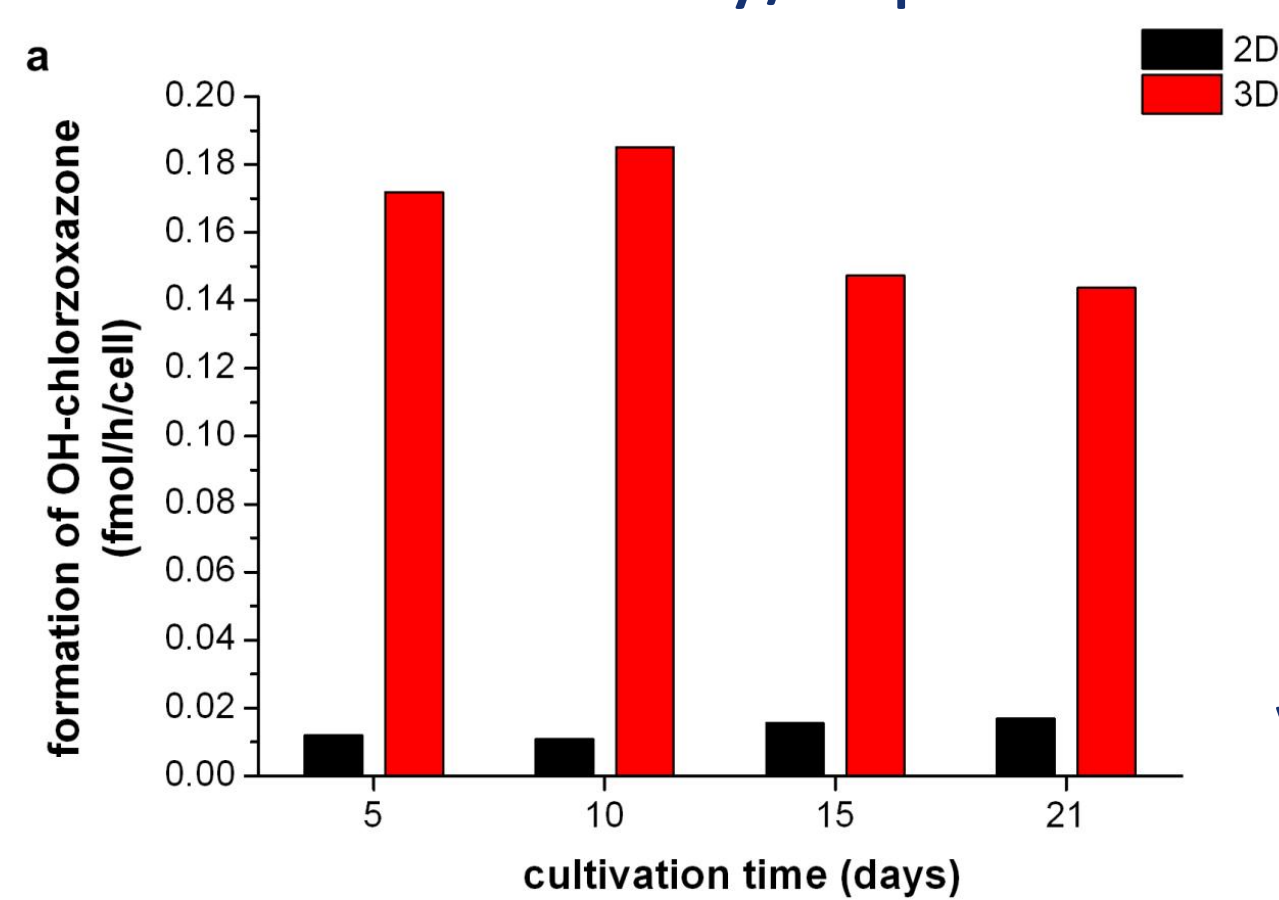


Figure 1: Formation of 3D HepaRG spheroids. HepaRG cells were seeded into GravityPlus plates (InSphero AG) at different densities (500, 2000 and 8000 cells).

CYP2E1 activity/expression



→ Higher CYP2E1 activity in the 3D vs. the 2D cultures

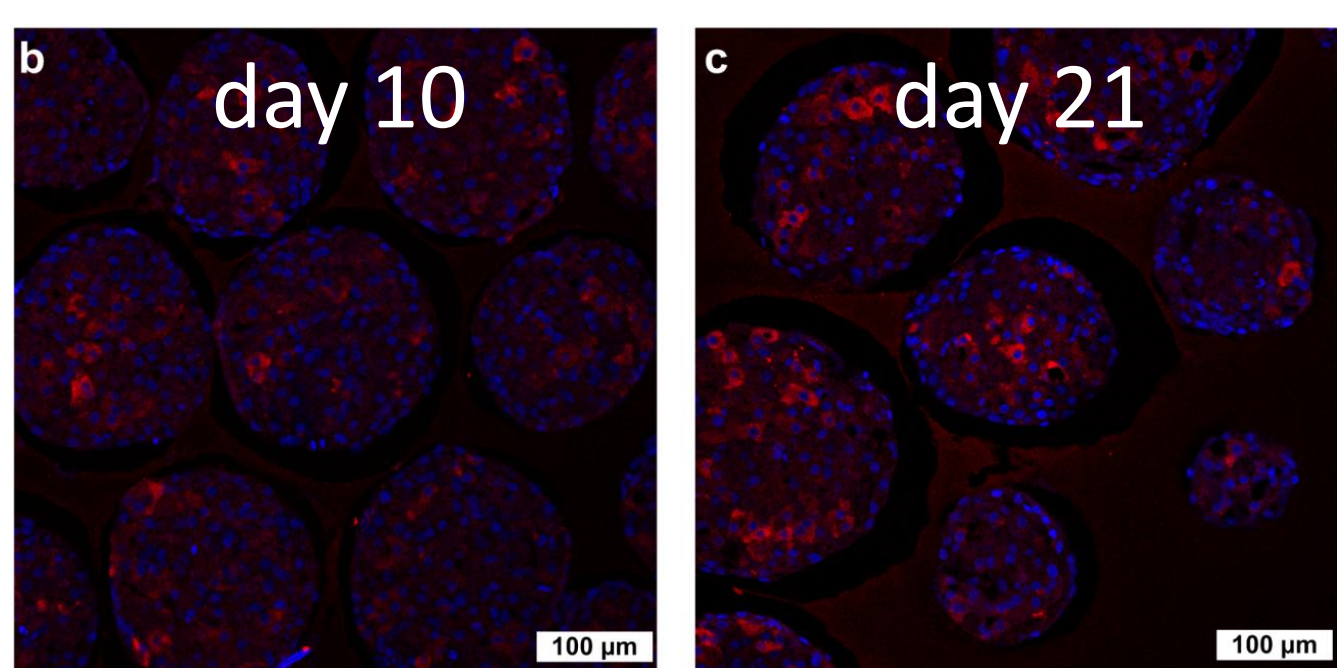


Figure 2: CYP2E1 enzyme activity in 2D and 3D and CYP2E1 protein expression in 3D HepaRG cultures.

Protein expression in 3D HepaRG spheroids

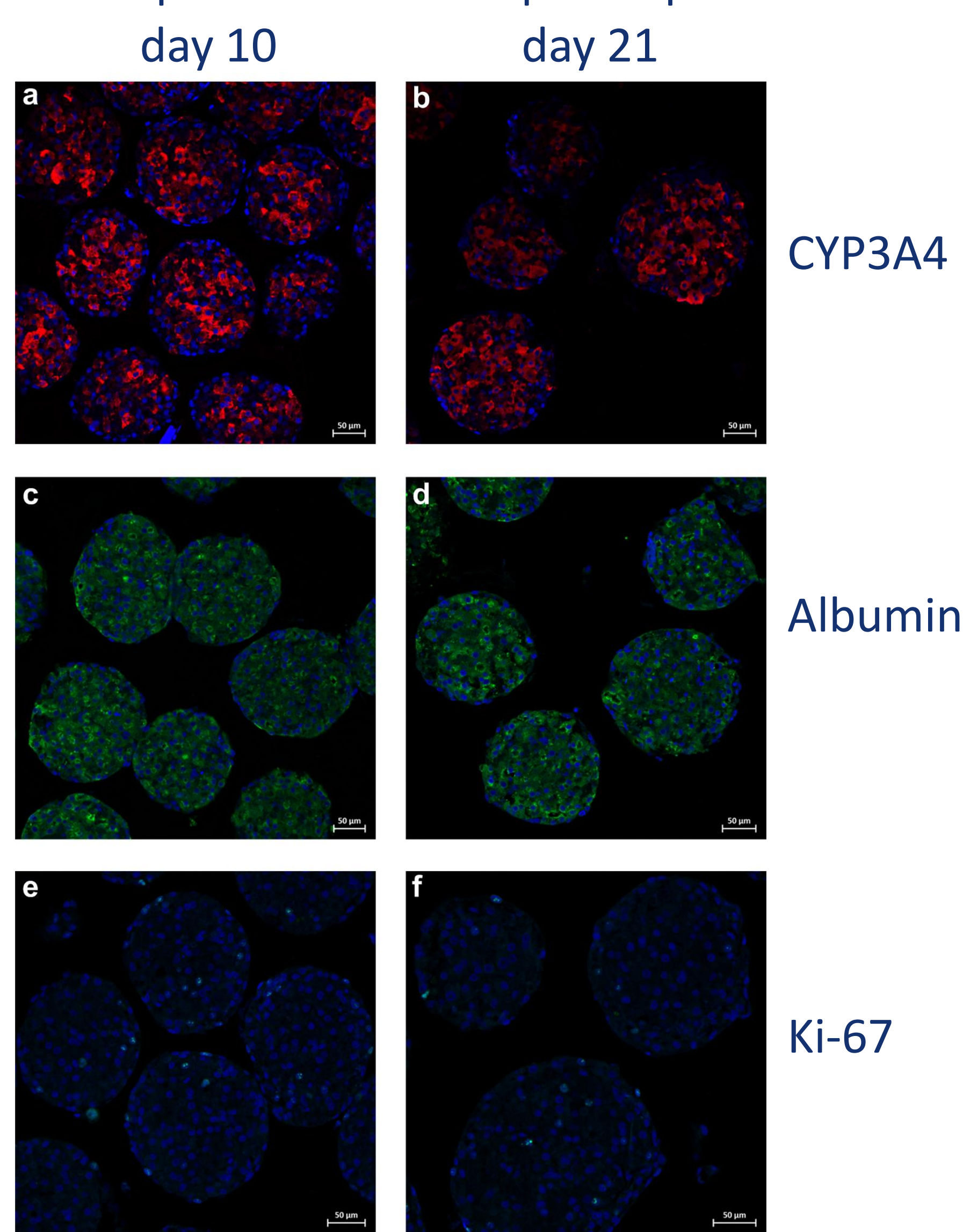


Figure 3: Protein expression in 3D HepaRG cultures. The expression of CYP3A4 (a, b), albumin (c, d) and Ki67 (e, f) in 3D HepaRG cultures was assessed by IHC. Scale bars represent 50 µm.

→ High expression of CYP3A4 and albumin in 3D cultures.
Low expression of the proliferation marker Ki-67.

MRP-2 activity / expression

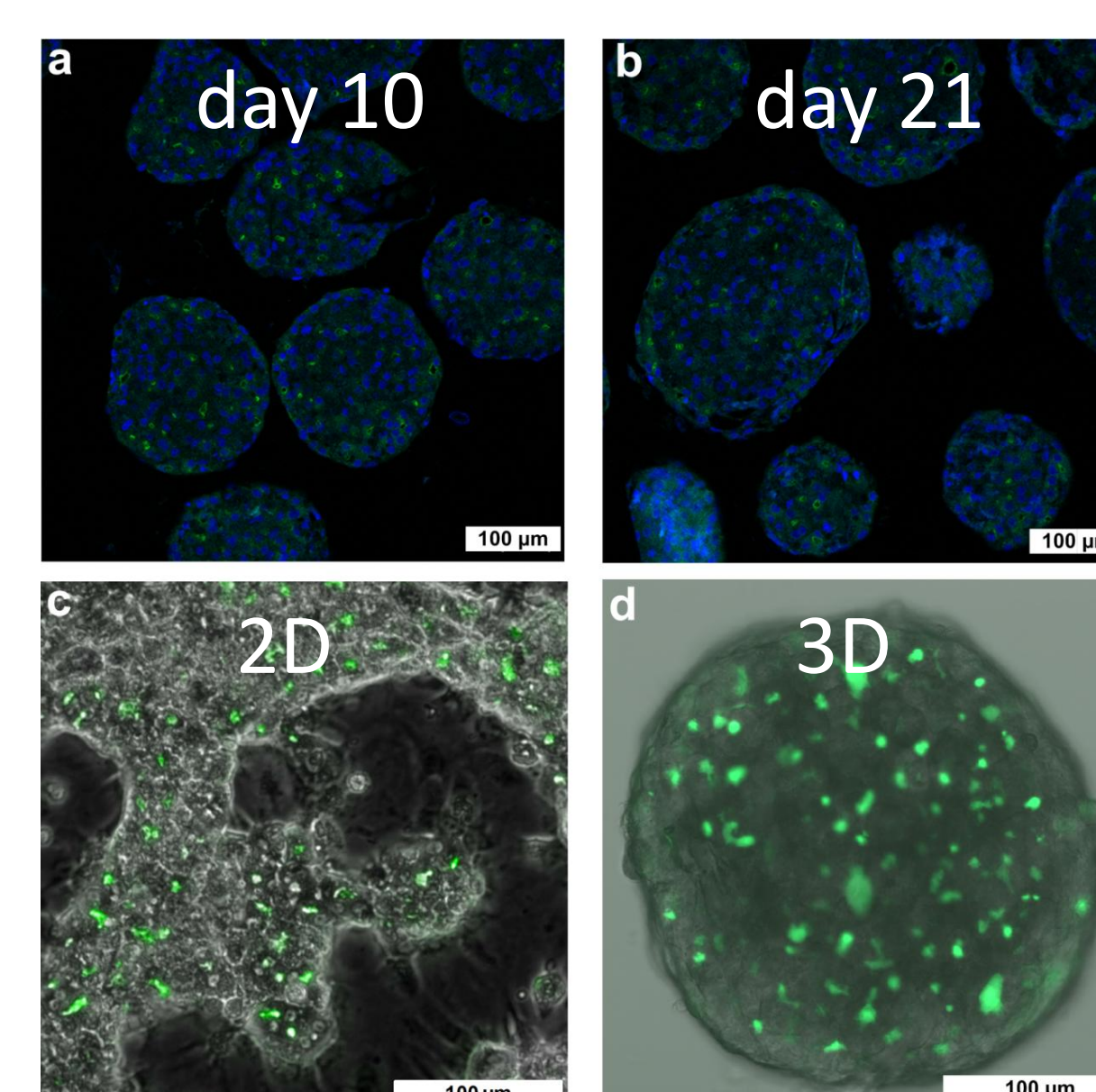


Figure 4: MRP2 transporter activity in 2D and 3D and MRP2 protein expression in 3D HepaRG cultures.

→ High expression of MRP-2

Acute toxicity of acetaminophen (APAP)

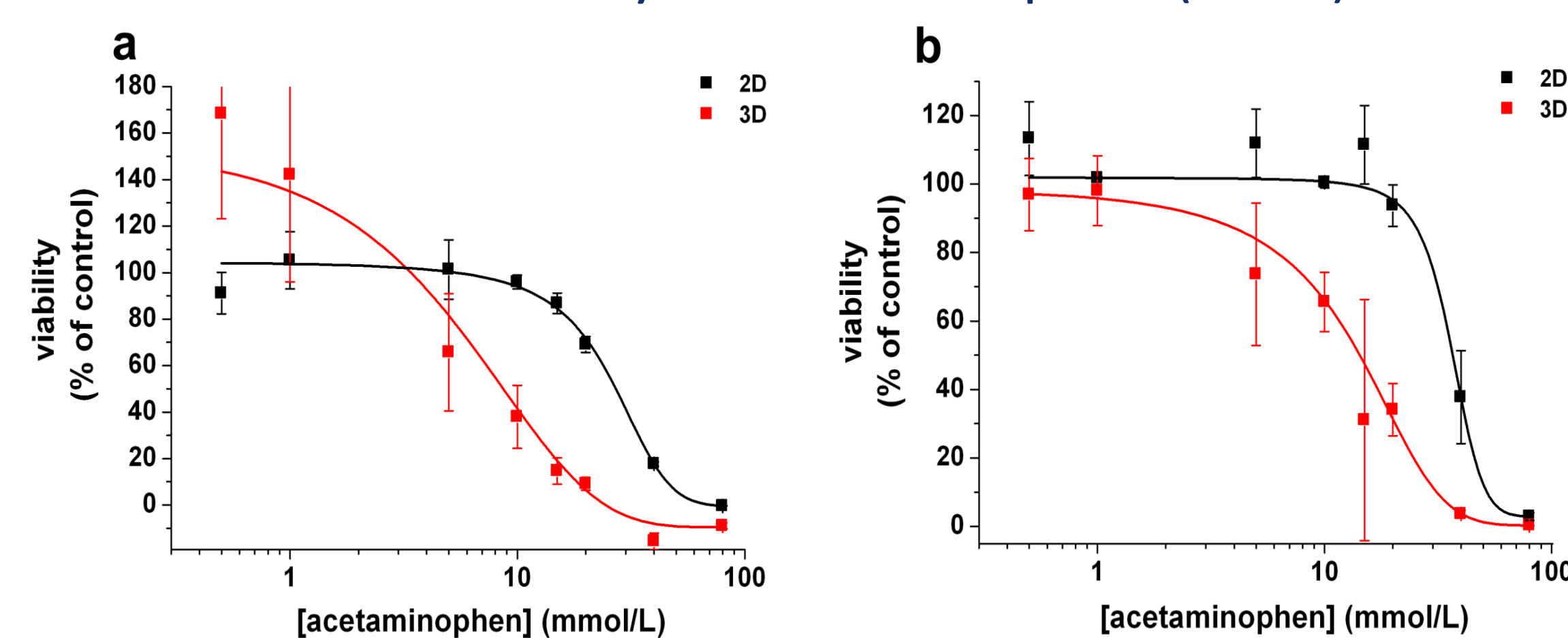


Figure 5: Acute toxicity of APAP in 2D and 3D HepaRG cultures, assessed on culture days 4 (a) or 21 (b).

→ 3D organotypic HepaRG cultures are more sensitive to APAP-induced hepatotoxicity than 2D cultures

Conclusions

- The 3D HepaRG organotypic cultures show higher CYP2E1 activity than 2D cultures. Moreover, they show high CYP3A4, albumin and MRP-2 activities during 3 weeks of cultivation. Only very low proliferation (no size increase, only little Ki67 expression) was observed.
- The 3D HepaRG organotypic cultures are more sensitive towards acetaminophen-induced hepatotoxicity than the 2D cultures.
- Our results suggest that the 3D HepaRG cultures are an excellent tool for toxicity studies as human-based *in vitro* alternative to animal tests.

Reference

Gunness P, Mueller E, Shevchenko V, Heinzle E, Ingelman-Sundberg M, Noor F (2013): 3D organotypic cultures of human HepaRG cells: a tool for in vitro toxicity studies. *Toxicological Sciences*, doi:10.1093/toxsci/ktf021

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