Comparison of 2D and 3D cultures of primary hepatocytes on hepatocellular functions and hepatotoxicity

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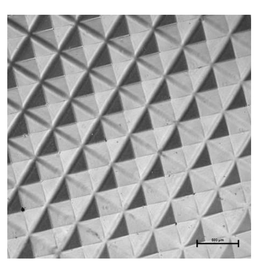
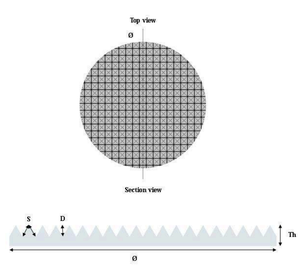
Background

- Primary mammalian hepatocytes are the system of choice for *in-vitro* applications as drug metabolism, toxicology, and transporter assays.
- Multiple *in-vitro* systems are available for 2D and 3D culture of various cell types
- Ferentis hydrogels characteristics:
  - Peptide hydrogel used as cell matrix for attachment and culture
  - CMP – collagen mimetic peptide as based material used (cell-repellent)
  - with molded tetrahedron microwell structure; dimensions: wide 400x400 µm, depth 350 µm -> increased cell-cell contacts in micro-wells, hydrogel diameter 10 mm for 48well plate





Objectives

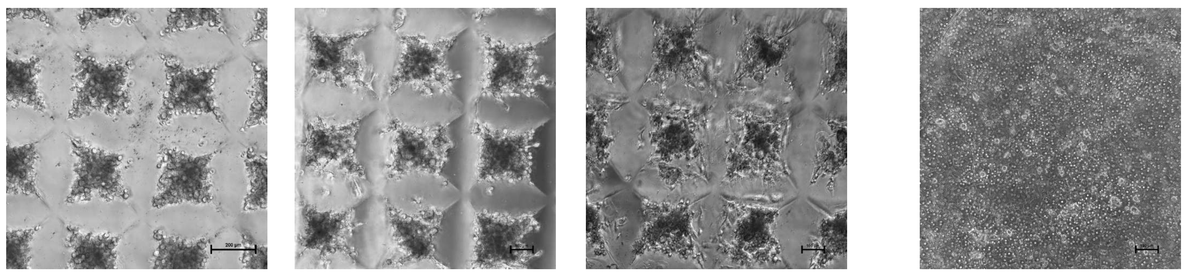
- Compare standard 2D culture on collagen-coated plates with:
  - new 3D matrix for hepatocytes culture: hydrogel discs consisting of collagen mimetic peptide
  - 3D hanging drop spheroid culture
- Analyse of hepatocellular vitality and functions (Urea release, Cytochrome P450 inducibility)
- Study the cell sensitivity for hepatotoxic substance Diclofenac (ATP content, LDH activity)

Methods

- Cryopreserved primary monkey (Cynomolgus) and dog (Beagle) hepatocytes from 3 donors per species were thawed and then cultured separately in 2D on collagen-coated plates (24well/ 96well) or in 3D on hydrogels (48well plate) or in spheroids (96well plate).
- Medium change: daily in 2D and 3D on hydrogel; every 2 days in spheroids
- Assays for hepatocellular functionality and toxicity were performed on the indicated days. Results were normalised to cell number or culture volume.

Results

3D culture on hydrogel vs. 2D



day 1 | day 3 | day 5 | day 3 – 2D

Fig. 1: Phase contrast microscopy of Cynomolgus hepatocytes in 3D on hydrogel (days 1/3/5) and 2D (day 3) in 48well plates.

- **Hepatocytes were settled in microwells of hydrogel, but spread on gel surface early in culture; 2D cultures showed typical polygonal hepatocytes**

Fig. 2: Urea release in Cynomolgus (A) and Beagle (B) hepatocytes.

- **Urea release was significantly higher in 3D hydrogel cultures compared to 2D cultures**

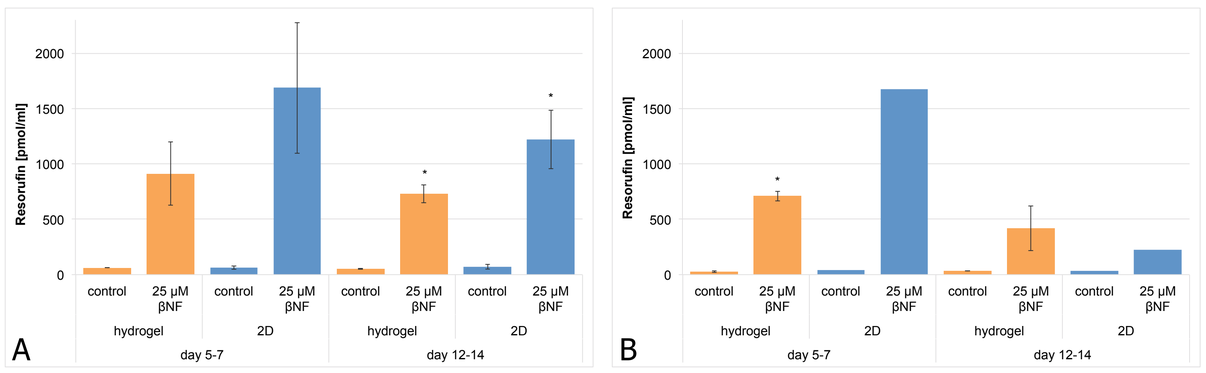


Fig. 3: EROD activity in Cynomolgus (A) and Beagle (B) hepatocytes, without normalisation to protein content.

- **High EROD induction at both time points and both species; induction factors in 3D < 2D**

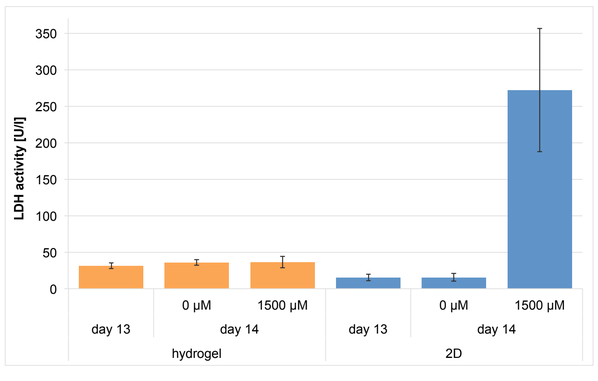


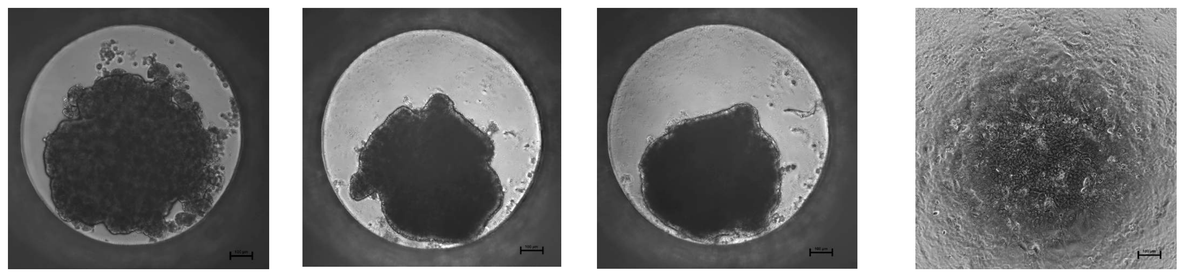
Fig. 4: LDH activity at treatment with hepatotoxin Diclofenac in Cynomolgus hepatocytes; similar results in Beagle hepatocytes observed (data not shown)

- **Cells did not react to Diclofenac treatment in 3D as they did in 2D**

Conclusions

- Major differences seem to exist between different 3D culture systems and in comparison to a standard 2D -> this may lead to conflicting results in drug toxicity assessment
- 3D cultures on cell-repellent hydrogels with microwells supported urea release and Cytochrome inducibility, but did not show typical compact spheroids in micro-wells and the cells did not react to Diclofenac
- Spheroid 3D cultures reacted to hepatotoxin, but Cytochrome 1A2 was only marginally inducible and they were not advantageous regarding detoxification functions (urea release)

3D culture in spheroids vs. 2D



day 3 | day 7 | day 13 | day 7 – 2D

Fig. 5: Phase contrast microscopy of Cynomolgus hepatocytes in 3D spheroids (days 3/7/13) and 2D (day 7) in 96well plates. Cell numbers used: 3D = 10000/well; 2D = 70000/well (Cynomolgus) or 90000/well (Beagle)

- **Few single small spheroids clustered with increasing culture time and formed one more condensed spheroid; 2D – differentiated hepatocytes**

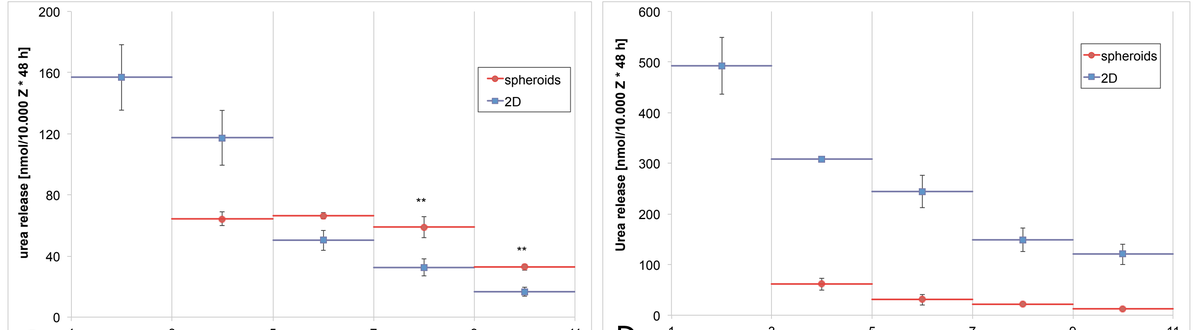


Fig. 6: Urea release in Cynomolgus (C) and Beagle (D) hepatocytes.

- **Urea release normalized to 10.000 cells/well; 2D culture is mostly advantageous for urea release compared to spheroid cultures**

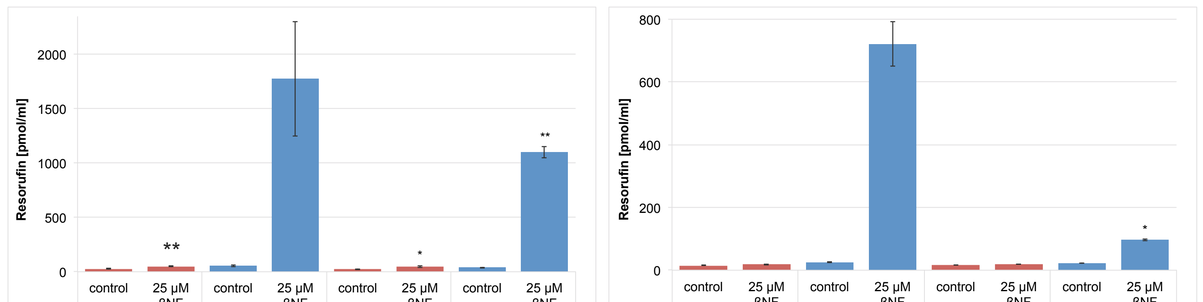


Fig. 7: EROD activity in Cynomolgus (C) and Beagle (D) hepatocytes, without normalisation to protein content.

- **Induction factor in 3D Cynomolgus hepatocytes: 2fold at both time points, in 2D 30fold; no induction in Beagle hepatocytes in 3D spheroids, in 2D 4-28fold**

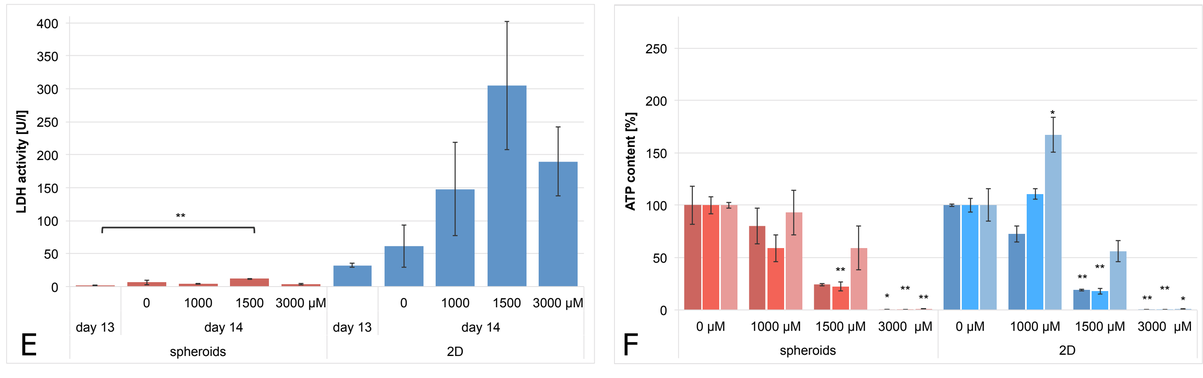


Fig. 8: LDH activity (E) and ATP content (F) in Cynomolgus hepatocytes

- **Diclofenac led to highest LDH activity at 1.5 mM in spheroids and 2D; ATP assay on viable cells showed significant reduction in ATP content with 1.5 mM Diclofenac in both culture systems**