**Background**

- Primary mammalian hepatocytes are the system of choice for in-vitro applications as drug metabolism, toxicity, 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 disc 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**

- Hepatocytes were settled in microwells of hydrogel, but spread on gel surface early in culture; 2D cultures showed typical polygonal hepatocytes
- Urea release was significantly higher in 3D hydrogel cultures compared to 2D

**3D culture in spheroids vs. 2D**

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

**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)

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

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