

Letter to the editor:

**ADVANCES IN 2D AND 3D IN VITRO SYSTEMS FOR
HEPATOTOXICITY TESTING**

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Dear Editor,

Currently, much enthusiasm surrounds the establishment of hepatocyte in vitro systems as alternatives for animal experiments (Parrott et al., 2011; Schaap et al., 2012; Hammad et al., 2013; Oloyede et al., 2013). It has been shown that hepatocytes under certain culture conditions form ‘microtissue’ with some features similar to the in vivo situation (Rago et al., 2009; Achilli et al., 2012; Messner et al., 2013). Moreover, precursor cells including embryonic stem cells can be differentiated to share some features with primary hepatocytes (Brulport et al., 2007; Aurich et al., 2009; Gabriel et al., 2012; Seeliger et al., 2013). The transcriptome of hepatocytes in vitro has been systematically compared to the in vivo situation (Godoy et al., 2010; Zellmer et al., 2010; Doktorova et al., 2012; Godoy and Bolt, 2012; Schug et al., 2013). Despite some differences response patterns of hepatocyte in vitro nevertheless give relevant insight into the toxic mode of action of chemicals (Hewitt et al., 2007; Bauer et al., 2009; Ullrich et al., 2009; Heise et al., 2012; Knobloch et al., 2012). Although there are large differences to primary hepatocytes, hepatoma derived cells still represent a useful and easy to handle system that can be helpful if one is aware of the limitations (Watanabe et al., 2011; Lin et al., 2012; Schreck et al., 2012; Tolosa et al., 2013). Last but not least, systems biology techniques have successfully supported the current progress in hepatotoxicity testing (Hoehme et al., 2007, 2010; Braeuning et al., 2010; Geenen et al., 2012). In this gold-rush mood currently prevailing in the field of hepatocyte in vitro systems an expert panel has recently published the probably most comprehensive review on the topic (Godoy et al., 2013). The more than 100-page article critically discusses the possibilities and limitations of liver in vitro systems with particular emphasis on hepatotoxicity testing. The reader learns how the physiological state of hepatocytes is altered when they are isolated from their in vivo microenvironment and are brought into culture (Godoy et al., 2009, 2010, 2013). Different culture systems and their advantages as well as limitations are critically reviewed, including monolayer cultures, sandwich cultures, co-cultures with non-parenchymal cells, spheroids or ‘microtissue’, liver slice cultures and the isolated perfused liver. A particular emphasis is given how to use these in vitro systems for studies of apoptosis and drug induced liver toxicity. Moreover, alternative hepatocyte sources, such as stem cell or hepatoma derived hepatocyte-like cells are critically discussed. The review of Godoy et al. (2013) is of high interest for anyone interested in liver physiology as well as hepatotoxicity testing.

REFERENCES

- Achilli TM, Meyer J, Morgan JR. Advances in the formation, use and understanding of multi-cellular spheroids. *Expert Opin Biol Ther* 2012;12:1347-60.
- Aurich H, Sgodda M, Kaltwasser P, Vetter M, Weise A, Liehr T et al. Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue in vitro promotes hepatic integration in vivo. *Gut* 2009;58:570-81.
- Bauer A, Schumann A, Gilbert M, Wilhelm C, Hengstler JG, Schiller J et al. Evaluation of carbon tetrachloride-induced stress on rat hepatocytes by ³¹P NMR and MALDI-TOF mass spectrometry: lysophosphatidylcholine generation from unsaturated phosphatidylcholines. *Chem Phys Lipids* 2009;159:21-9.
- Braeuning A, Singh Y, Rignall B, Buchmann A, Hammad S, Othman A et al. Phenotype and growth behavior of residual β -catenin-positive hepatocytes in livers of β -catenin-deficient mice. *Histochem Cell Biol* 2010;134:469-81.
- Brulport M, Schormann W, Bauer A, Hermes M, Elsner C, Hammersen FJ et al. Fate of extrahepatic human stem and precursor cells after transplantation into mouse livers. *Hepatology* 2007;46:861-70.
- Doktorova TY, Ellinger-Ziegelbauer H, Vinken M, Vanhaecke T, van Delft J, Kleinjans J et al. Comparison of genotoxin-modified transcriptomic responses in conventional and epigenetically stabilized primary rat hepatocytes with in vivo rat liver data. *Arch Toxicol* 2012;86:1703-15.
- Gabriel E, Schievenbusch S, Kolossov E, Hengstler JG, Rotshteyn T, Bohlen H et al. Differentiation and selection of hepatocyte precursors in suspension spheroid culture of transgenic murine embryonic stem cells. *PLoS One* 2012;7:e44912.
- Geenen S, Taylor PN, Snoep JL, Wilson ID, Kenna JG, Westerhoff HV. Systems biology tools for toxicology. *Arch Toxicol* 2012;86:1251-71.
- Godoy P, Bolt HM. Toxicogenomic-based approaches predicting liver toxicity in vitro. *Arch Toxicol* 2012;86:1163-4.
- Godoy P, Hengstler JG, Ilkavets I, Meyer C, Bachmann A, Müller A et al. Extracellular matrix modulates sensitivity of hepatocytes to fibroblastoid dedifferentiation and transforming growth factor beta-induced apoptosis. *Hepatology* 2009;49:2031-43.
- Godoy P, Lakkapamu S, Schug M, Bauer A, Stewart JD, Bedawi E et al. Dexamethasone-dependent versus -independent markers of epithelial to mesenchymal transition in primary hepatocytes. *Biol Chem* 2010;391:73-83.
- Godoy P, Hewitt NJ, Albrecht U, Andersen ME, Ansari N, Bhattacharya S et al. Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. *Arch Toxicol* 2013;87:1315-30.
- Hammad S, Marchan R, Hengstler JG. Cutting-edge topics in research on animal sciences. *JEAAS* 2013;1:1-3.
- Heise T, Schug M, Storm D, Ellinger-Ziegelbauer H, Ahr HJ, Hellwig B et al. In vitro - in vivo correlation of gene expression alterations induced by liver carcinogens. *Curr Med Chem* 2012;19:1721-30.
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Hewitt NJ, Lechón MJ, Houston JB, Hallifax D, Brown HS, Maurel P et al. Primary hepatocytes: current understanding of the regulation of metabolic enzymes and transporter proteins, and pharmaceutical practice for the use of hepatocytes in metabolism, enzyme induction, transporter, clearance, and hepatotoxicity studies. *Drug Metab Rev* 2007;39:159-234.

Hoehme S, Hengstler JG, Brulport M, Schaefer M, Bauer A, Gebhardt R et al. Mathematical modelling of liver regeneration after intoxication with CCl₄. *Chem Biol Interact* 2007;168:74-93.

Hoehme S, Brulport M, Bauer A, Bedawy E, Schormann W, Hermes M et al. Prediction and validation of cell alignment along microvessels as order principle to restore tissue architecture in liver regeneration. *Proc Natl Acad Sci USA* 2010;107:10371-6.

Knobeloch D, Ehnert S, Schyschka L, Büchler P, Schoenberg M, Kleeff J et al. Human hepatocytes: isolation, culture, and quality procedures. *Methods Mol Biol* 2012;806:99-120.

Lin J, Schyschka L, Mühl-Benninghaus R, Neumann J, Hao L, Nussler N et al. Comparative analysis of phase I and II enzyme activities in 5 hepatic cell lines identifies Huh-7 and HCC-T cells with the highest potential to study drug metabolism. *Arch Toxicol* 2012;86:87-95.

Messner S, Agarkova I, Moritz W, Kelm JM. Multi-cell type human liver microtissues for hepatotoxicity testing. *Arch Toxicol* 2013;87:209-13.

Oloyede GK, Adaramoye OA, Oguntokun OJ. Phytochemical and hepatotoxicity studies on *Adansonia digitata* leaf extracts. *JEAAS* 2013;1:25-34.

Parrott JL, Kohli J, Sherry JP, Hewitt LM. In vivo and in vitro mixed-function oxygenase activity and vitellogenin induction in fish and in fish and rat liver cells by stilbenes isolated from scotch pine (*Pinus sylvestris*). *Arch Environ Contam Toxicol* 2011;60:116-23.

Rago AP, Chai PR, Morgan JR. Encapsulated arrays of self-assembled microtissues: an alternative to spherical microcapsules. *Tissue Eng Part A* 2009;15:387-95.

Schaap MM, Zwart EP, Wackers PF, Huijskens I, van de Water B, Breit TM et al. Dissecting modes of action of non-genotoxic carcinogens in primary mouse hepatocytes. *Arch Toxicol* 2012;86:1717-27.

Schreck I, Deigendesch U, Burkhardt B, Marko D, Weiss C. The *Alternaria* mycotoxins alternariol and alternariol methyl ether induce cytochrome P450 1A1 and apoptosis in murine hepatoma cells dependent on the aryl hydrocarbon receptor. *Arch Toxicol* 2012;86:625-32.

Schug M, Stöber R, Heise T, Mielke H, Gundert-Remy U, Godoy P et al. Pharmacokinetics explain in vivo/in vitro discrepancies of carcinogen-induced gene expression alterations in rat liver and cultivated hepatocytes. *Arch Toxicol* 2013;87:337-45.

Seeliger C, Culmes M, Schyschka L, Yan X, Damm G, Wang Z et al. Decrease of global methylation improves significantly hepatic differentiation of Ad-MSCs: possible future application for urea detoxification. *Cell Transplant* 2013;22:119-31.

Tolosa L, Gómez-Lechón MJ, Pérez-Cataldo G, Castell JV, Donato MT. HepG2 cells simultaneously expressing five P450 enzymes for the screening of hepatotoxicity: identification of bioactivable drugs and the potential mechanism of toxicity involved. *Arch Toxicol* 2013;87:1115-27.

Ullrich A, Stolz DB, Ellis EC, Strom SC, Michalopoulos GK, Hengstler JG et al. Long term cultures of primary human hepatocytes as an alternative to drug testing in animals. *ALTEX* 2009;26:295-302.

Watanabe T, Ohta Y, Mizumura A, Kobayashi Y, Hirano S. Analysis of arsenic metabolites in HepG2 and AS3MT-transfected cells. *Arch Toxicol* 2011;85:577-88.

Zellmer S, Schmidt-Heck W, Godoy P, Weng H, Meyer C, Lehmann T et al. Transcription factors ETF, E2F, and SP-1 are involved in cytokine-independent proliferation of murine hepatocytes. *Hepatology* 2010;52:2127-36.