Primary hepatocyte cultures for liver disease modeling

Vinken, Mathieu

Published in:
Current Opinion in Toxicology

DOI:
10.1016/j.cotox.2020.08.003

Publication date:
2021

Primary hepatocyte cultures for liver disease modeling

Mathieu Vinken

Department of *In Vitro* Toxicology and Dermato-Cosmetology, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium.

Correspondence: Mathieu Vinken, Ph.D., Pharm.D., E.R.T., Department of *In Vitro* Toxicology and Dermato-Cosmetology, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium; E-mail: mathieu.vinken@vub.be; Tel: +32 2 477 45 87.

Word count (excluding references): 1617.

Highlights:

- Liver disease research relies to an important extent on the use of *in vitro* models.
- Interdisciplinary collaboration will increase the translational value of primary hepatocyte cultures.

Abstract

Liver disorders constitute a worldwide increasing problem. Both the study of the mechanisms underlying liver disease and the development of liver disease therapeutics heavily rely on the use of experimental models. Among those, human-based *in vitro* systems are more and more preferred over laboratory animals because of ethical reasons. Primary human hepatocytes and their cultures are still considered as the gold standard liver-based *in vitro* models, as they provide a good reflection of the *in vivo* situation. Nevertheless, these *in vitro* systems deal with the gradual deterioration of the differentiated morphological and functional phenotype. This can be overcome by following a number of strategies, such as by using spheroid/organoid and sandwich culture configurations. Further improvement in this area in view of enhancing overall translational value of primary human hepatocytes and their cultures warrants interdisciplinary collaboration.

Key words: liver, disease, *in vitro*, primary hepatocyte.

At least 650 million people worldwide are affected by some form of liver disorder. In Europe, the prevalence of liver disease was estimated to be approximately 6%, which equals to about 29 million people. The average European mortality rate for chronic liver pathologies is 14.3 per 100.000 habitants, making it the fifth most common cause of death in Europe. This means that more than 70.000 Europeans are dying from chronic liver disease every year [1,2]. The European economic burden of liver disease is substantial and has been estimated at €645 per patient per month. Hospitalizations account for 50.6% of the overall direct costs per month, with 41.2% being attributed to treatment. In addition, patients and family caregivers lose on average 1.15 days of productivity per patient per month [1]. The only curative therapy for liver failure is transplantation. More than 5.500 liver transplantations per year are currently performed in Europe, costing up to €100.000 the first year and €10.00€ yearly thereafter [1,3]. Drug-related hepatotoxicity accounts for more than 50% of acute liver failure cases [4]. More than 1000 drugs have been associated with drug-induced liver injury, especially of the cholestatic type, including anti-infectious drugs, anti-diabetics, anti-inflammatory drugs, psychotropic drugs, cardiovascular drugs and steroids [5]. Drug-induced liver injury is a major reason of drug failure during pre-marketing and post-marketing phases, accounting for up to 29% of all drug withdrawals [6]. Acute liver failure is responsible for 6% of all liver-related deaths and for 7% of all liver transplantations in Europe [7].

Driven by the epidemiologic relevance as well as the ubiquitous lack of efficient therapies, many teams, both in academic and industrial settings, have devoted their research to the characterization of appropriate druggable targets and/or the development of new liver disease therapeutics, but equally to the establishment of strategies to predict liver toxicity induced by drugs. Animal models play a pivotal role throughout these research efforts. A wide variety of animal models, mostly in rodents, is currently used for studying liver diseases, including acute liver failure [8], cholestatic disorders [9], non-alcoholic steatohepatitis [10], liver fibrosis and

cirrhosis [11], and different types of liver cancer [12]. They are typically based on the use of specific chemicals, well-defined diets, genetic modifications, surgical procedures or infection-based strategies. Besides obvious ethical constraints and high costs, such animal models only reproduce some aspects of the corresponding human pathology because of interspecies differences. In this respect, it is well-known that a mere 50% of clinical human drug-induced liver injury cases can be predicted in rodents [13]. Even for seemingly generic processes, such as inflammation, the underlying mechanisms show poor correlation between rodents and human [14]. For these reasons, and in particular in view of increasing translational value, considerable attention has been paid, and is still being paid, to the use of human liver-based *in vitro* models. A vast number of state-of-the-art human-based *in vitro* models to study liver disease is available today. They can be roughly grouped in 2 main classes, namely liver-derived *in vitro* models and stem cell-derived *in vitro* models [15,16]. The latter have emerged over the past 2 decades and involve stem cells of various origin that can be differentiated *in vitro* to hepatocyte-like cells by exposure to specific cytokines and growth factors, thereby mimicking liver embryogenesis. Hepatocyte-like cells derived from stem cells have been found a promising model of liver steatotic disorders [17,18]. A major breakthrough in this field came with the introduction of the induced pluripotent stem cell technique [19]. However, although some groups showed their relevance [20,21], liver cells obtained from induced pluripotent stem cells are not the most appropriate *in vitro* systems for cholestasis research [22]. In general, great promise lies with stem cell-derived *in vitro* models for studying liver disease. Nevertheless, major challenges still need to be tackled, such as related to the efficiency of differentiation protocols and the homogeneity of resulting hepatocyte-like cell populations as well as their expression of the full repertoire of *in vivo*-relevant functional features.

At present, liver-derived *in vitro* models seem better fit for liver disease modeling. They are diverse in nature, and range from precision-cut human liver slices, primary human hepatocytes

and human liver-derived cell lines all up to subcellular human liver fractions [15,16]. All these liver-based *in vitro* models differ in complexity and longevity, and have their own specific strengths and flaws (Table 1).

Precision-cut human liver slices maintain liver architecture and thus overall cell-cell as well as cell-extracellular matrix interactions. Accordingly, they provide a good reflection of the hepatic *in vivo* situation [23,24]. Precision-cut human liver slices have been shown appropriate for studying cholestatic disorders [25]. The preparation of precision-cut human liver slices requires quite some technical skills. Moreover, the viability of precision-cut human liver slices is limited to a couple of days, therefore impeding the study of long-term effects [15].

Human-based liver cell lines are typically derived from liver cancers, in particular hepatocellular carcinoma. Because of their growth capacity, they provide a virtually unlimited cell supply [26]. Cell lines are easy to use and typically yield highly reproducible testing results. However, although exceptions exist, such as holds for the human hepatoma HepaRG cell line that has a good biotransformation and drug transporter capacity [27], most liver cell lines show aberrant functionality and morphology due to their carcinogenic origin, which jeopardizes *in vivo* relevance [15]. Nonetheless, liver cell lines have shown their value in studying liver steatosis [28] and cholestasis [29].

Subcellular liver fractions are popular systems because of their ease of use. Among those are microsomes and S9 factions, which are obtained by centrifugating human liver homogenates at 100.000*g* and 9.000*g*, respectively. Microsomes contain most of the hepatocyte's endoplasmic reticulum, including cytochrome P450 biotransformation enzymes. For this reason, microsomes are abundantly used in early drug development, in particular for metabolite profiling and cytochrome P450 biotransformation enzyme inhibition and induction studies [30]. However, their applicability for investigating liver disease is minimal, as their composition strongly deviates from *in vivo* liver cyto-architecture [15].

Isolated primary hepatocytes and their cultures are still considered as the gold standard liver-based *in vitro* model, because they provide a good reflection of the hepatic *in vivo* situation. Nonetheless, they cope with the gradual loss of the differentiated phenotype at the functional and morphological level. This so-called dedifferentiation process is triggered during the isolation of primary hepatocytes from human liver, which routinely relies on a 2-steps collagenase perfusion technique. By consecutively abolishing cell-cell and cell-extracellular matrix interactions, proliferative and inflammatory responses are initiated, both that drastically suppress the expression of liver-specific factors, such as cytochrome P450 biotransformation enzymes. This dedifferentiation event starts within minutes after the start of the isolation procedure and progressively manifests upon subsequent cultivation of hepatocytes, which limits their use for long-term testing purposes [15,16]. A number of strategies has been introduced to counteract hepatocyte dedifferentiation in an attempt to set up culture systems that can be applied for extended periods of time. Such anti-dedifferentiation strategies typically strive to re-establish the *in vivo* micro-environment of hepatocytes. One of those approaches includes the restoration of cellular contacts by culturing primary hepatocytes in a tridimensional configuration in so-called spheroids [31] (Figure 1). This may even be combined by co-culturing of primary hepatocytes with other liver cell types, whether or not in the presence of a scaffold, yielding organoids [32]. Primary hepatocytes, either freshly isolated or cryopreserved, in spheroid culture functionally express liver-specific features, including cytochrome P450 biotransformation enzymes and drug transporters, for several weeks. They have been shown to be eminent systems for studying cholestasis [33], liver fibrosis [34] and steatosis [35]. Spheroid cultures of primary human hepatocytes can monitor delayed cholestatic effects, and can distinguish between adversity and homeostatic adaptation [33]. Furthermore, they allow to pick up interindividual differences in responses towards disease and toxicity, which is highly clinically relevant [36]. Another anti-dedifferentiation strategy includes the cultivation of

primary human hepatocytes between 2 layers of extracellular matrix components, such as collagen. Such sandwich culture systems enable primary hepatocytes to regain their polarity and cyto-architecture, which in turn favors their functional capacity [37] (Figure 1). As holds for spheroid cultures, sandwich cultures of primary human hepatocytes can be used for several weeks, in particular for investigating cholestatic events [38].

In recent years, the primary hepatocyte users and research field has witnessed several new exciting developments to enhance longevity and functionality of freshly isolated or cryopreserved primary hepatocytes in culture, thus increasing their translational value. Among those are genetic [26] and epigenetic [39] modifications, both that alter gene transcription of primary hepatocytes in favor of the differentiated phenotype. A more pragmatic approach includes the combination of anti-dedifferentiation strategies, and, in particular, the involvement of expertise other than cell biology. Such interdisciplinary efforts are key to advance this field forward. Of specific interest is the area of bio-engineering, which has already resulted in a number of advanced liver-based *in vitro* models, namely human liver bioreactors [40] and human liver chips/microphysiological systems [41], by implementing state-of-the-art technological features, such as microfluidics and in-built sensors to monitor hepatocyte functionality and disease responses in real-time. Nonetheless, many challenges still lie ahead, including providing such state-of-the-art *in vitro* models with microbiota, which have emerged in the past few years as major players in liver disease [42,43]. Another aspect that deserves further scrutiny includes the accurate modeling of interindividual susceptibility towards liver disease and toxicity. This is critical to predict idiosyncratic drug-induced liver injury [44], and more in general to advance personalized toxicology/medicine. Research in these directions in the upcoming years, including induced pluripotent stem cell technology [45], should be strongly encouraged and will not only necessitate further interdisciplinary efforts, but equally intersectoral collaboration, in particular between academia and (pharmaceutical) industry.

Acknowledgements

This work was financially supported by the grants of the Willy Gepts Fonds, the Fund for Scientific Research-Flanders, Cosmetics Europe and the Johns Hopkins University Center for Alternatives to Animal Testing USA.

Declarations of interest

None.

References

* Of special interest.

** Of outstanding interest.

[1] M. Blachier, H. Leleu, M. Peck-Radosavljevic, D.C. Valla, F. Roudot-Thoraval, The burden of liver disease in Europe: a review of available epidemiological data. J. Hepatol. 58 (2013) 593-608.

[2] United European Gastroenterology, Report on the research symposium on digestive and liver diseases, September 18, 2012, European Parliament, Brussels; 1-15.

[3] M. van Agthoven, H.J. Metselaar, H.W. Tilanus, R.A. de Man, J.N. IJzermans, B.M. Martin van Ineveld, A comparison of the costs and effects of liver transplantation for acute and for chronic liver failure. Transpl. Int. 14 (2001) 87-94.

[4] D.S. Goldberg, K.A. Forde, D.M. Carbonari, J.D. Lewis, K.B. Leidl, K.R. Reddy, K. Haynes, J. Roy, D. Sha, A.R. Marks, J.L. Schneider, B.L. Strom, D.A. Corley, V. Lo Re 3rd, Population-representative incidence of drug-induced acute liver failure based on an analysis of an integrated health care system. Gastroenterology 148 (2015) 1353-1361.

[5] K.R. Bhamidimarri, E. Schiff, Drug-induced cholestasis. Clin. Liver Dis. 17 (2013) 519-531.

[6] W.M. Lee, Drug-induced acute liver failure. Clin. Liver Dis. 17 (2013) 575-586.

[7] A.M. Larson, J. Polson, R.J. Fontana, T.J. Davern, E. Lalani, L.S. Hynan, J.S. Reisch, F.V. Schiødt, G. Ostapowicz, A.O. Shakil, W.M. Lee, Acetaminophen-induced acute liver failure: results of a United States multicenter prospective study. Hepatology 42 (2005) 1364-1372.

[8] M. Maes, M. Vinken, H. Jaeschke, Experimental models of hepatotoxicity related to acute liver failure. Toxicol. Appl. Pharmacol. 290 (2016) 86-97.

[9] V. Mariotti, M. Cadamuro, C. Spirli, R. Fiorotto, M. Strazzabosco, L. Fabris L., Animal models of cholestasis: an update on inflammatory cholangiopathies. Biochim. Biophys. Acta Mol. Basis Dis. 1865 (2019) 954-964.

[10] J. Willebrords, I.V. Pereira, M. Maes, S. Crespo Yanguas, I. Colle, B. Van Den Bossche, T.C. Da Silva, C.P. de Oliveira, W. Andraus, V.A. Alves, B. Cogliati, M. Vinken, Strategies, models and biomarkers in experimental non-alcoholic fatty liver disease research. Prog. Lipid Res. 59 (2015) 106-125.

[11] S.C. Yanguas, B. Cogliati, J. Willebrords, M. Maes, I. Colle, B. van den Bossche, C.P.M.S. de Oliveira, W. Andraus, V.A.F. Alves, I. Leclercq, M. Vinken, Experimental models of liver fibrosis. Arch. Toxicol. 90 (2016) 1025-1048.

[12] Z.J. Brown, B. Heinrich, T.F. Greten, Mouse models of hepatocellular carcinoma: an overview and highlights for immunotherapy research. Nat. Rev. Gastroenterol. Hepatol. 15 (2018) 536-554.

[13] Z. Liu Z, Q. Shi, D. Ding, R. Kelly, H. Fang, W. Tong, Translating clinical findings into knowledge in drug safety evaluation: drug induced liver injury prediction system (DILIps). PLoS Comput. Biol. 7 (2011) e1002310.

[14] J. Seok, H.S. Warren, A.G. Cuenca, M.N. Mindrinos, H.V. Baker, W. Xu, D.R. Richards, G.P. McDonald-Smith, H. Gao, L. Hennessy, C.C. Finnerty, C.M. López, S. Honari, E.E. Moore, J.P. Minei, J. Cuschieri, P.E. Bankey, J.L. Johnson, J. Sperry, A.B. Nathens, T.R. Billiar, M.A. West, M.G. Jeschke, M.B. Klein, R.L. Gamelli, N.S. Gibran, B.H. Brownstein, C. Miller-Graziano, S.E. Calvano, P.H. Mason, J.P. Cobb, L.G. Rahme, S.F. Lowry, R.V. Maier, L.L. Moldawer, D.N. Herndon, R.W. Davis, W. Xiao, R.G. Tompkins, Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc. Natl. Acad. Sci. USA 110 (2013) 3507-3512.

** [15] V. Vilas-Boas, A. Cooreman, E. Gijbels, R. Van Campenhout, E. Gustafson, S. Ballet, P. Annaert, B. Cogliati, M. Vinken, Primary hepatocytes and their cultures for the testing of drug-induced liver injury. Adv. Pharmacol. 85 (2019) 1-30.

This paper gives an in-depth overview of primary hepatocyte cultures, their strengths, flaws and applications.

[16] J. Fraczek, J. Bolleyn, T. Vanhaecke, V. Rogiers, M. Vinken, Primary hepatocyte cultures for pharmaco-toxicological studies: at the busy crossroad of various anti-dedifferentiation strategies. Arch. Toxicol. 87 (2013) 577-610.

[17] R.M. Rodrigues, J. De Kock, S. Branson, M. Vinken, K. Meganathan, U. Chaudhari, A. Sachinidis, O. Govaere, T. Roskams, V. De Boe, T. Vanhaecke, V. Rogiers, Human skin-derived stem cells as a novel cell source for *in vitro* hepatotoxicity screening of pharmaceuticals. Stem Cells Dev. 23 (2014) 44-55.

[18] A. Pradip, D. Steel, S. Jacobsson, G. Holmgren, M. Ingelman-Sundberg., P. Sartipy, P. Björquist, I. Johansson, J. Edsbagge, High-content analysis of human pluripotent stem cell-derived hepatocytes reveals drug-induced steatosis and phospholipidosis. Stem Cells Int. 2016 (2016) 2475631.

[19] K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126 (2006) 663-676.

[20] A. Ghodsizadeh, A. Taei, M. Totonchi, A. Seifinejad, H. Gourabi, B. Pournasr, N. Aghdami, R. Malekzadeh, N. Almadani, G.H. Salekdeh, H. Baharvand, Generation of liver disease-specific induced pluripotent stem cells along with efficient differentiation to functional hepatocyte-like cells. Stem Cell Rev. 6 (2010) 622-632.

[21] K. Imagawa, K. Takayama, S. Isoyama, K. Tanikawa, M. Shinkai, K. Harada, M. Tachibana, F. Sakurai, E. Noguchi, K. Hirata, M. Kage, K. Kawabata, R. Sumazaki, H. Mizuguchi, Generation of a bile salt export pump deficiency model using patient-specific induced pluripotent stem cell-derived hepatocyte-like cells. Sci. Rep. 7 (2017) 41806.

[22] C.C. Bell, V.M. Lauschke, S.U. Vorrink, H. Palmgren, R. Duffin, T.B. Andersson, M. Ingelman-Sundberg, Transcriptional, functional, and mechanistic comparisons of stem cell-derived hepatocytes, HepaRG Cells, and three-dimensional human hepatocyte spheroids as predictive in vitro systems for drug-induced liver injury. Drug Metab. Dispos. 45 (2017) 419-429.

[23] E. Palma, E.J. Doornebal, S. Chokshi, Precision-cut liver slices: a versatile tool to advance liver research. Hepatol. Int. 13 (2019) 51-57.

[24] E. Bigaeva, E. Gore, E. Simon, M. Zwick, A. Oldenburger, K.P. de Jong, H.S. Hofker, M. Schlepütz, P. Nicklin, M. Boersema, J.F. Rippmann, P. Olinga, Transcriptomic characterization of culture-associated changes in murine and human precision-cut tissue slices. Arch. Toxicol. 93 (2019) 3549-3583.

[25] S. Vatakuti, P. Olinga, J.L.A. Pennings, G.M.M. Groothuis, Validation of precision-cut liver slices to study drug-induced cholestasis: a transcriptomics approach. Arch. Toxicol. 91 (2017) 1401-1412.

[26] E. Ramboer, T. Vanhaecke, V. Rogiers, M. Vinken, Immortalized human hepatic cell lines for *in vitro* testing and research purposes. Methods Mol. Biol. 1250 (2015) 53-76.

[27] C. Aninat, A. Piton, D. Glaise, T. Le Charpentier, S. Langouët, F. Morel, C. Guguen-Guillouzo, A. Guillouzo, Expression of cytochromes P450, conjugating enzymes and nuclear receptors in human hepatoma HepaRG cells. Drug Metab. Dispos. 34 (2006) 75-83.

[28] L. Tolosa, M.J. Gómez-Lechón, N. Jiménez, D. Hervás, R. Jover, M.T. Donato, Advantageous use of HepaRG cells for the screening and mechanistic study of drug-induced steatosis. Toxicol. Appl. Pharmacol. 302 (2016) 1-9.

[29] C. Guguen-Guillouzo, A. Guillouzo, Set-up and use of HepaRG cells in cholestasis research. Methods Mol. Biol. 1981 (2019) 291-312.

[30] H. Zhang, C.D. Davis, M.W. Sinz, A.D. Rodrigues, Cytochrome P450 reaction-phenotyping: an industrial perspective. Expert Opin. Drug Metab Toxicol. 3 (2007) 667-687.

[31] T.B. Andersson, Evolution of novel 3D culture systems for studies of human liver function and assessments of the hepatotoxicity of drugs and drug candidates. Basic Clin. Pharmacol. Toxicol. 121 (2017) 234-238.

** [32] H. Hu, H. Gehart, B. Artegiani, C. Löpez-Iglesias, F. Dekkers, O. Basak, J. van Es, S.M. Chuva de Sousa Lopes, H. Begthel, J. Korving, M. van den Born, C. Zou, C. Quirk, L. Chiriboga, C.M. Rice, S. Ma, A. Rios, P.J. Peters, Y.P. de Jong, H. Clevers, Long-term expansion of functional mouse and human hepatocytes as 3D organoids. Cell 175 (2018) 1591-1606.

This work shows the long-term cultivation of primary human hepatocytes in organoid culture.

[33] D.F. Hendriks, L. Fredriksson Puigvert, S. Messner, W. Mortiz, M. Ingelman-Sundberg, Hepatic 3D spheroid models for the detection and study of compounds with cholestatic liability. Sci. Rep. 6 (2016) 35434.

** [34] T. Hurrell, V. Kastrinou-Lampou, A. Fardellas, D.F.G. Hendriks, Å Nordling, I. Johansson, A. Baze, C. Parmentier, L. Richert, M. Ingelman-Sundberg, Human liver spheroids as a model to study aetiology and treatment of hepatic fibrosis. Cells 9 (2020) 964.

This work shows the use of spheroid cultures of primary human hepatocytes for studying liver fibrosis.

** [35] M. Kozyra, I. Johansson, Å Nordling, S. Ullah, V.M. Lauschke, M. Ingelman-Sundberg, Human hepatic 3D spheroids as a model for steatosis and insulin resistance. Sci. Rep. 8 (2018) 14297.

This work shows the use of spheroid cultures of primary human hepatocytes for studying liver steatosis.

* [36] C. Parmentier, D.F.G. Hendriks, B. Heyd, P. Bachellier, M. Ingelman-Sundberg, L. Richert, Interindividual differences in the susceptibility of primary human hepatocytes towards drug-induced cholestasis are compound and time dependent. Toxicol. Lett. 295 (2018) 187-194.

This work shows the use of spheroid cultures of primary human hepatocytes for studying interindividual susceptibility towards cholestasis.

[37] K. Yang, C. Guo, J.L. Woodhead, R.L. St Claire 3rd, P.B. Watkins, S.Q. Siler, B.A. Howell, K.LR. Brouwer, Sandwich-cultured hepatocytes as a tool to study drug disposition and drug-induced liver injury. J. Pharm. Sci. 105 (2016) 443-459.

* [38] N. Deferm, L. Richert, P. Van Brantegem, T. De Vocht, B. Qi, P. de Witte, T. Bouillon, P. Annaert, Detection of drug-induced cholestasis potential in sandwich-cultured human hepatocytes. Methods Mol. Biol. 1981 (2019) 335-350.

This work shows the use of sandwich cultures of primary human hepatocytes for studying cholestasis.

[39] J. Bolleyn, J. Fraczek, V. Rogiers, T. Vanhaecke, Epigenetic modifications as antidedifferentiation strategy for primary hepatocytes in culture. Methods Mol. Biol. 1250 (2015) 203-211.

[40] S.P. Rebelo, R. Costa, M.F. Sousa, C. Brito, P.M. Alves, Establishing liver bioreactors for *in vitro* research. Methods Mol. Biol. 1250 (2015) 189-202.

* [41] A. Ehrlich, D. Duche, G. Ouedraogo,Y. Nahmias, Challenges and opportunities in the design of liver-on-chip microdevices. Annu. Rev. Biomed. Eng. 21 (2019) 219-239.

This paper gives an overview of the set-up and use of liver chips/microphysiological systems.

[42] C.A. Philips, P. Augustine, P.K. Yerol, G.N. Ramesh, R. Ahamed, S. Rajesh, T. George, S. Kumbar, Modulating the intestinal microbiota: therapeutic opportunities in liver disease. J. Clin. Transl. Hepatol. 8 (2020) 87-99.

* [43] K.J. Schwenger, N. Clermont-Dejean, J.P. Allard, The role of the gut microbiome in chronic liver disease: the clinical evidence revised. JHEP Rep. 1 (2019) 214-226.

This paper gives an overview of the role of microbiota in liver disease.

[44] L. Tolosa, N. Jiménez, G. Pérez, J.V. Castell, M.J. Gómez-Lechón, M.T. Donato, Customised *in vitro* model to detect human metabolism-dependent idiosyncratic drug-induced liver injury. Arch. Toxicol. 92 (2018) 383-399.

[45] V.L. Dao Thi, X. Wu, R.L. Belote, U. Andreo, C.N. Takacs, J.P. Fernandez, L.A. Vale-Silva, S. Prallet, C.C. Decker, R.M. Fu, B. Qu, K. Uryu, H. Molina, M. Saeed, E. Steinmann, S. Urban, R.R. Singaraja, W.M. Schneider, S.M. Simon, C.M. Rice, Stem cell-derived polarized hepatocytes. Nat. Commun. 11 (2020) 1677.

Figure and table legends

Figure 1: Spheroid/organoid and sandwich cultures of primary human hepatocytes.

Table 1: Liver-based *in vitro* models.

Figure 1

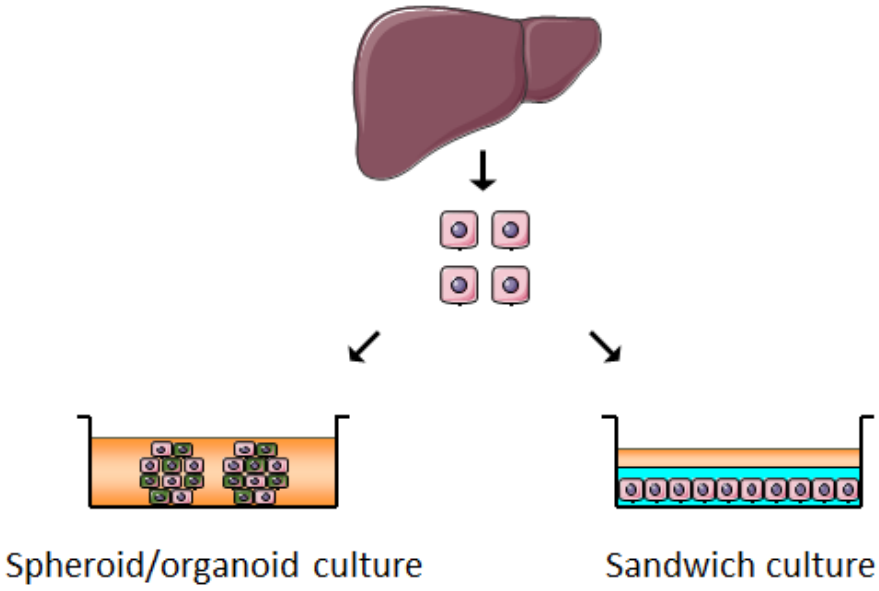

Table 1

Strenghts	Flaws	Translational value
Precision-cut human liver slices		
Preservation of liver structure	- Requires high technical skills - Limited viability	+++
Primary human hepatocyte cultures		
May be cryopreserved	- No preservation of liver structure - Cope with progressive dedifferentiation	+++
Human liver-derived cell lines		
- Unlimited cell supply - Highly reproducible test results	- Originate from 1 single donor - May be dedifferentiated	+
Subcellular human liver fractions		
- Readily available - Easy to use	No preservation of liver structure	-