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THE ADVANTAGES AND LIMITATIONS OF HUMAN LIVER MODELS IN *IN VITRO* TOXICOLOGY STUDY

Abstract: This review collects and analyses information about modern liver models used in toxicological studies from 2015 to January 2026. Currently, human liver models are increasingly being improved and various approaches being explored, including the selection of different cell types and advanced cultivation methods, to more accurately simulate the *in vivo* liver environment to replace animal models. These new methods are increasing the complexity of *in vitro* systems. However, the complex liver models currently available are not yet a complete replacement for animal models and does not have all the characteristics of the liver. Since many models undergo phenotypic changes during prolonged cultivation, this limits their suitability for long-term studies of repeated administration toxicity. Moreover, they have limited suitability, lack of vascularization, labour demanding and expensive. However, compared to early 2D hepatocyte cultures, these modern systems demonstrate significantly improved prediction accuracy and physiological relevance. With continued development, it may be possible to create models capable of tracking the occurrence, progression, and potential reversibility of liver drug toxicity.
Keywords: liver cells, HepG2, HepaRG, 2D culture, 3D culture, toxicology.

Introduction

The liver is the central organ for the metabolism and transformation of xenobiotics and is therefore a major focus of toxicological research. The toxicological effect of drugs is one of the main reasons for drug elimination during development (Guengerich, 2011). Moreover, proper liver models are also used to study mechanism of action of different environmental pollutions (Baratzhanova et al., 2025). This highlights the need for reliable preclinical models capable of predicting hepatotoxicity. Although animal models have traditionally been used to assess safety, interspecies differences and ethical considerations have stimulated the development of human-relevant *in vitro* systems (Poh & Stanslas, 2024).

The use of human cell culture systems makes it possible to study human metabolism and physiology, which are difficult to accurately reproduce in *in vivo* studies (Philippeos et al., 2012). Cell culture mimics the reaction of target tissue cells in the human body in response to chemicals. However, there are important differences between systems of whole organisms and isolated cellular systems regarding the types and complexity of toxic effects that can be measured. In animal studies, toxicity assessment often focuses on one or two critical endpoints that appear first when the dose of a xenobiotic is increased. Although such models provide valuable systemic information, they may not reflect the full range of biological reactions occurring in the body, and some effects may go unnoticed during experimental observation. In contrast, cell culture models allow the assessment of specific cellular and molecular mechanisms of toxicity. Nevertheless, the results obtained *in vitro* require careful verification of compliance with the *in vivo* data obtained in humans to confirm their physiological significance.

In vitro systems are particularly advantageous for screening purposes because localized toxicity can be assessed more quickly under controlled exposure conditions. In addition, cellular toxicity data can be

compared with calculated *in vivo* exposure levels to improve risk assessment. The other advantage of cell culture models is the relative homogeneity of the cell population, which reduces variability resulting from genetic and environmental factors. This makes possible to manipulate genes and molecular pathways. It allows generation of data with high reproducibility and consistency, which cannot be guaranteed when studying entire organ systems (Segeritz & Vallier, 2017).

Nowadays, significant progress has been made in the development and application of cellular systems, which has led to the stability and functionality of liver models. An important and main criteria for evaluating liver models in fundamental or pharmacological studies is their ability to perform basic liver functions and maintain appropriate metabolic pathways. As mentioned above, liver plays a central role in the metabolism of endogenous substrates and exogenous compounds, the regulation of homeostasis of amino acids, carbohydrates and lipids, the synthesis of plasma proteins such as albumin and transferrin, as well as in the activation of inflammatory and immune responses after damage caused by diseases, drugs or toxic effects (Zeilinger et al., 2016).

Hepatocytes are generally considered to be metabolically adequate models for *in vitro* assessment of hepatotoxicity. They are increasingly being used in studies on xenobiotic toxicity, including genotoxic effects (Guo et al., 2020). Over the past decades, liver modelling has progressed from traditional two-dimensional (2D) hepatocyte cultures to more advanced three-dimensional (3D) systems, including spheroids, organoids, extracellular matrix 3D models, and organs on a chip. These models aim to more accurately reproduce the structural and functional complexity of the human liver. However, increasing the complexity of the model does not always lead to improved predictive characteristics, and cultivation conditions, endpoints, and applications vary significantly (Fukunaga & Takebe, 2025).

Given this rapid development of liver modelling technologies and their growing role in toxicological assessment, it is necessary to conduct a comprehensive analysis of the available models and their applications. The purpose of this review study is to systematically examine existing *in vitro* human liver models used in toxicology, summarize their advantages and limitations, and identify gaps that need to be addressed to increase their predictive value regarding the toxicity of xenobiotics to the liver.

Data collection method

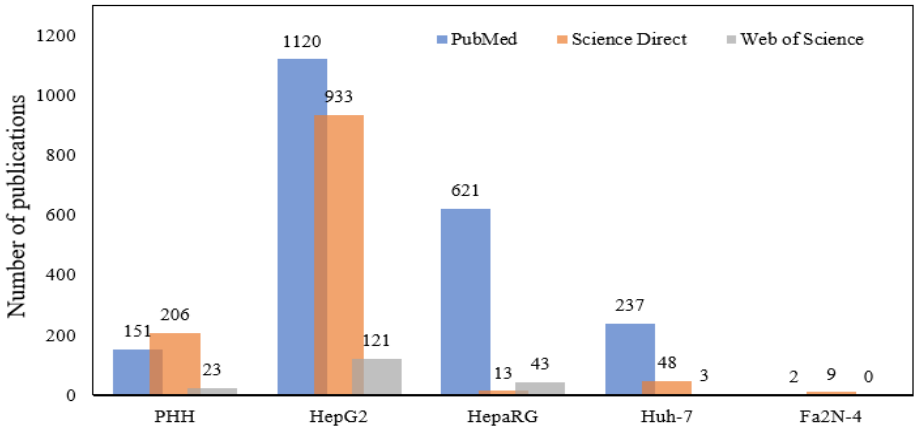
This scoping review was conducted to map existing and new literature on hepatocyte models which can be used in toxicology studies. A comprehensive literature search was conducted through online library sources in PubMed, Science Direct, Web of Science from 2015 to January 2026. The specific search criteria for Science Direct and Web of Science was (“2D liver cells” OR “3D liver cells” OR “liver cell” OR “HepG2” OR “HepaRG” OR “PHH liver cell” OR “HuH-7 “) AND (“toxicology research” OR “ drug discovery”). For PubMed search criteria was (“Primary human hepatocytes” OR “HepG2” OR “HepaRG” OR “HuH-7” OR “Fa2N-4”) AND (toxicolog* OR toxico* OR “toxicity” OR “toxic effects” OR “toxic response”). Key words such as “2D hepatocyte models”, “3D hepatocyte models”, “toxicology research on liver cells”, etc. were used.

Reference list of included studies was manually screened to identify other relevant articles. Included articles were describing or evaluating hepatocyte-based models used in toxicology studies. Only studies published in English were included. The results were summarized in a descriptive form. Hepatocyte models were classified according to their structural complexity (2D, 3D, organoids, etc). Research trends, advantages, limitations, and gaps in the literature were summarized in Tables and Figures.

Results

The database search yielded 518 articles in Web of Science and 4761 articles for Science Direct, and 1424 for PubMed. Search depending on liver cell types was also performed and showed that among three databases article concerning usage of HepG2 in toxicological studies was predominant (Fig.1). After removing duplicate, articles were screened by title and abstract.

Figure 1
Literature search results for human liver cell publications across three databases (from January 2015 to January 2026)



2D cultures

Primary human hepatocytes (PHH) isolated from the liver have morphological and biochemical characteristics that are most similar to the original tissue (Segeritz & Vallier, 2017). Such cells are considered the gold standard for the creation of human-appropriate models for the cultivation of liver cells *in vitro*. They recreate the functionality of the liver as *in vivo*, and provide fairly accurate results in pharmacological and toxicological studies *in vitro*. Also, these cells allow analysing a wide range of genetic polymorphisms, since they are derived from an individual donor. However, this fact also complicates model standardization due to interindividual variability and alterations induced during isolation procedure. In addition, the limited availability and complex logistical requirements associated with PHH cells prevent their large-scale application (Shulman & Nahmias, 2013; Zeilinger et al., 2016). The characteristics of the donor, including age, liver fat content and pre-existing liver damage, as well as procedural factors such as tissue conditions and transportation time, significantly affect cell viability (Richert et al., 2004). Immediately after tissue extraction, hepatocytes become very sensitive to ischemic damage and quickly lose their viability. Regardless of preservation method there is usually a significant decrease in the activity of enzymes that metabolize phase I and phase II drugs (Godoy et al., 2013; Kammerer & Küpper, 2018). These disadvantages makes them also expensive to use (Van den Hof et al., 2014).

Several approaches have been proposed to overcome the limited cell viability of PHH (Castell et al., 2006). One such strategy involves cellular immortalisation. Immortalized cell lines are characterized by their ability to proliferate indefinitely *in vitro*, overcoming normal replicative senescence. This state is usually achieved through genetic modifications such as ectopic expression of telomerase reverse transcriptase (hTERT) or introduction of viral oncogenes that suppress key cell cycle regulators (e.g., p53 and Rb), or by culturing tissues derived from tumours.

Viral oncogene introduction. Transformed Human Liver Epithelial-2 cells (THLE-2), derived from healthy human liver, were immortalized by the introduction of the simian virus 40 large T antigen (SV40) (Pfeifer et al., 1993). Yalda Soltanpour *et al.* in their study analysed the expression of 96 representative genes involved in drug absorption, distribution, metabolism, and excretion of drugs (ADME) in THLE-2 cells. These genes encode various transporters, phase I and II drug-metabolizing enzymes, and nuclear hormone receptors (Table 1). During their study, it was shown that the expression of most genes was absent or at a low level, with the exception of some cytochrome P450 (CYP) genes, CYP2C9 and CYP3A4 (Soltanpour et al., 2012).

Other human hepatocytes immortalised by transfection with SV40 are Fa2N-4 cells. They were derived from a 12-year-old female donor. These cells show ability to induce *CYP3A4, CYP2C9, CYP1A2, UGT1A,* and MDR1 mRNA in response to known in-

ducers similar to PHH. Moreover, this induction also can be noticed at the enzyme activity level (Mills et al., 2004). Youdim *et al.* confirmed that Fa2N-4 cells are suitable model for evaluation of P450 induction using cocktail approach (Youdim et al., 2007). Moreover, they are easily available and can be grown in culture as needed for experimental use, unlike freshly isolated human hepatocytes, whose availability depends on access to suitable donor liver tissue (Mills et al., 2004). Hariparsad *et al.* showed that the expression of PXR and AhR is similar between Fa2N-4 cells and human hepatocytes, but CAR and some hepatic uptake transporters, the organic anion-transporting polypeptides, were significantly reduced in this cell line compared to primary cells (Hariparsad et al., 2008).

Hepatocarcenoma cell lines. Considerable efforts were made to develop reliable cell models of human liver cells that would show a fairly accurate prediction of results after exposure to genotoxic chemicals for effective screening. As a result, several models of human hepatocarcinoma cell cultures were created, which are used in toxicological studies, like HepG2 (Knasmüller et al., 2004), HepaRG (Mandon et al., 2019), HuH-7 (Nakabayashi et al., 1982), etc (Table 1). Cells obtained from liver tumour tissue have been widely used as models for toxicological studies *in vitro*. These cell lines have good availability, high ability to proliferate and stable metabolism. However, the high potential for proliferation of these cells usually leads to the loss of differentiated functions. Because of this, for toxicological studies, it is necessary to take into account the specific functional characteristics of the cell lines (Zeilinger et al., 2016).

HepG2 cell line, derived from hepatocellular carcinoma, was obtained from a 15-year-old Caucasian male diagnosed with hepatoblastoma. This cell line has been extensively characterised and retains a number of functional properties of normal hepatocytes (Roe et al., 1993). HepG2 cells are cheap and easy to cultivate, and over the years, a significant amount of experimental data has been accumulated, allowing for the creation of an extensive reference database (Figure 1) (Mišík et al., 2019). Moreover, HepG2 cells are less technically demanding than PHH and demonstrate superior experimental reproducibility (Ramirez et al., 2018). However, there have been still some reports of showing interlaboratory variability, as well as inconsistency in the reproducibility of experiments with individual compounds (Waldherr et al., 2018).

These cells express a wide range of CYP enzymes; However, both mRNA and protein levels are generally lower than those observed in normal hepatocytes. In addition, they are also capable of performing second-phase metabolic reactions, including glutathione conjugation as well as glucuronidation and sulfation reactions (Donato et al., 2008). Notably, HepG2 cells exhibit relatively high levels of antioxidant enzymes and intracellular glutathione compared to many other human cell lines. Similar metabolic responses to dioxin and dibenzofurans have been reported in HepG2 and PHH (Dehn et al., 2005). In addition, transcriptomic analysis showed that HepG2 cells exhibit increased expression of genes involved in DNA, RNA, and protein metabolism, as well as cell cycle-related processes compared to HepaRG and PHH cells (Jennen et al., 2010).

HepG2 cells have been shown to be suitable for cytotoxicity screening by assessing reactive oxygen species (ROS) formation, glutathione depletion, and loss of membrane integrity (Schoonen et al., 2005). In addition, comparable modulation of gene expression profiles was observed in both PHH and HepG2 cells after exposure to the hepatotoxic compound trovafloxacin (Liguori et al., 2008). Magkoufopoulou *et al.* further showed that the integration of transcriptome analysis with the Ames test improves the prediction of genotoxic potential *in vitro* (Magkoufopoulou et al., 2012). Moreover, transcriptomic profiling of HepG2 cells exposed to toxic substances allowed the development of an *in vitro* screening test for phospholipidosis (Sawada et al., 2005). Van den Hof *et al.* applied gene expression analysis in HepG2 cells to classify chemical compounds according to their hepatotoxic properties (Van den Hof et al., 2014).

HepG2 often used as well to study human Apolipoprotein-B100 metabolism because they secretes predominately higher density Apolipoprotein-B100 -containing particles and low density lipoproteins, unlike normal human liver, which secretes Apolipoprotein-B100 mainly associated with VLDL particles (Meex et al., 2011). In the study of Meex *et al.*, they showed that HepG2 cells are more efficient in lipidation of apoB100 than Huh-7 cells. Compared to PHH, HepG2 cells has defect in assembly of hepatic VLDL. Tsai *et al.* showed that through inhibition of the overactive ras-MEK-ERK pathway this defect can be corrected (Tsai et al., 2007).

HepG2 cells also wildly used model to predict mitochondrial dysfunction and hepatotoxicity (Berger et al., 2016; Kamalian et al., 2015; Ramirez et al., 2018; Stanley & Wolf, 2022). Unlike many mammalian cells, HepG2 cells exhibit metabolic flexibility, allowing them to switch between glycolysis and oxidative phosphorylation to sustain proliferation, even

under low-oxygen conditions. This capacity to rely on glycolysis can render them less sensitive to mitochondrial toxicants (Marroquin et al., 2007). However, Kamalian *et al.* demonstrated that HepG2 cells subjected to acute glucose deprivation become more dependent on mitochondrial respiration and therefore represent a suitable model for detecting mitochondrial toxicity. The combined assessment of ATP levels and cytotoxicity in both metabolic states provides a more comprehensive evaluation of the underlying mechanisms of toxicity (Kamalian et al., 2015).

Huh-7 cells is a well-differentiated and characterized *in vitro* model of hepatocellular carcinoma (HCC) that is widely used in metabolic research. The cell line was isolated in 1982 from 57-year-old man with highly differentiated HCC and was proposed as an alternative to PHH and HepG2 cells (Nakabayashi et al., 1982). Huh-7 cells are often used to evaluate the efficacy and metabolism of anticancer drugs that affect the liver. For example, Huh-7 cells have proven valuable for studying drug distribution in the liver mediated by transporter proteins and for studying interactions with proteins associated with multidrug resistance (Jouan et al., 2016; Malinen et al., 2019). In addition, Huh-7 cells have also been effectively used in toxicity studies and are capable of bioactivating certain compounds, including acetaminophen, which leads to the formation of the hepatotoxic metabolite *N*-acetyl-*p*-benzoquinone imine (Nelson et al., 2015). Jouan *et al.* showed that Huh-7 cells exhibit significant activity of the multidrug-resistant protein (MRP), which probably reflects the expression of several liver MRPs, including MRP2. However, these cells lack the functional activity of key uptake transporters such as organic anion-transporting polypeptides, sodium-taurocholate co-transporting polypeptide, and organic cation transporter 1, as well as tubular transporters including P-glycoprotein and breast cancer resistance protein (Jouan et al., 2016).

Huh-7 cells have several distinctive features. In particular, they are homozygous for the null CYP3A5*3 null allele, indicating that the metabolism of CYP3 substrates is mediated exclusively by CYP3A4 (Dorr et al., 2017). Another characteristic is that Huh-7 cells secreted more Apolipoprotein-B100 than HepG2 cells did, according to the mass data (normalized to cell protein) (Meex et al., 2011). HuH-7 cells are also highly susceptible to the hepatitis C virus (HCV) which make them perfect model to study HCV (Lohmann et al., 1999). However, genomic analyses revealed significant chromosomal abnormalities in Huh-7 cells, including changes affecting all chromosomes and extensive loss of heterozygosity covering approximately 55.3% of the genome, consistent with the presence of numerous homozygous variants identified by sequencing (Kasai et al., 2018).

HepaRG cell line was derived from the liver tumour of a patient with chronic hepatitis C and macronodular cirrhosis (Marion et al., 2010). In culture, HepaRG cells typically differentiate into a mixed population of hepatocyte-like cells and cholangiocyte-like cells (Aninat et al., 2006). These cells retain the ability to express a wide range of liver-specific genes involved in carbohydrate, bile acid, and lipid metabolism, as well as in xenobiotic detoxification processes (Guo et al., 2020). HepaRG cells express key drug transporters such as ATP-binding cassettes (ABCs), nuclear receptors (e.g., CAR and PXR), CYP enzymes, phase II conjugating enzymes, and functional proteins such as albumin, transferrin, aldolase B, as well as various apical and tubular proteins (Zheng et al., 2021). It is important to note that differentiated HepaRG cells are considered one of the most comparable *in vitro* liver models to PHH (Cerec et al., 2007).

It was shown that genes located in 7th chromosome are highly expressed in HepaRG cells in comparison to PHH due to extra copy of this chromosome (Hart et al., 2010). Complex proteomic and secretomic analyses have shown that HepaRG cells have functional mitochondria and retain the ability to secrete hepatokines (Tascher et al., 2019). Compared to HepG2 cells, HepaRG cells exhibit higher activity of CYP enzymes and phase II conjugation enzymes. The differences in expression levels in the major drug-response gene families, such as CYPs, ALDHs, ADHs, NATs, FMOs, GSTs, UGTs, SULTs, ABCBs, ABCCs, ABCGs, and SLCOs showed that HepG2 and PHH were have much larger differences than the differences between HepaRG and PHH for most genes (Hart et al., 2010). In addition, HepaRG cells have been shown to be more sensitive to detecting DNA damage caused by xenobiotics, which confirms their suitability for genotoxicity assessment (Guo et al., 2020; Seo et al., 2019, 2020).

However, HepaRG cells less sensitive in detecting some direct- and indirect acting genotoxic carcinogens, such as cadmium chloride, etoposide, 2-amino-3-methylimidazo, and 2,4-diaminotoluene (Mandon et al., 2019; Mišík et al., 2019). Another limitation of this cells are their expansivity, scarce, and recurrence in higher levels of maintenance (Donato et al., 2013).

HepaRG cells are highly proliferative, they can differentiate toward hepatocytic and biliary lineage and into functional hepatocytes *in vivo*. Moreover,

probably due to their tumour origin, HepaRG cells have the potential to transdifferentiate through a hepatic bipotent progenitor (Cerec et al., 2007). It was also shown that HepaRG cells have higher galactose/sorbitol elimination rates and albumin production in comparison to PHH, however urea production was not observed. As well CYP expression was found to be more stable over cultivation in HepaRG cells (Lübberstedt et al., 2011).

Human Induced pluripotent stem cells (iPSCs). Recently human iPSCs became popular a renewable starting material for generating primary cell types, as they enable the recapitulation of human tissue from a sustainable source (Gracey & Lampe, 2025). In liver research, most teams focus on differentiating iPSCs into hepatocyte-like cells (HLC) that will replicate the metabolic activity of PHH. Multiple studies have demonstrated the applicability of HLC in drug metabolism studies, toxicity screening and modelling of specific liver diseases (Dao Thi et al., 2020; Donowitz et al., 2020; Gómez-Lechón & Tolosa, 2016; Mann, 2015; Matsui et al., 2020). Nevertheless, full maturation and restoration of HLC metabolic activity to adult levels remains a challenge, and none of the existing protocols for differentiating iPSCs into HLCs can produce cells that fully replicate the phenotype of adult human liver (Graffmann et al., 2022).

Table 1
Overview of 2D liver cells used in toxicology studies

Models	Advantages	Disadvantages	Application	References
PHH	+ Functionality of the liver as *in vivo* + Diversity in genetic polymorphisms	– Limited availability – Instability of the phenotype – Short time cultivation – Higher Cost	• Hepatotoxicity • DILI prediction • Inter-individual variability test	(Parmentier et al., 2018; Segeritz & Vallier, 2017; Shulman & Nahmias, 2013; Zeilinger et al., 2016)
THLE-2	+ Non-tumorigenic + Expression of CYP2C9 and CYP3A4 genes + Stable cell culture	– Expression of ADME genes is absent or in low level – Low receptor abundance	• Oxidative stress • inflammatory responses • Cytotoxic assays	(Sefried et al., 2018; Shah et al., 2014; Soltanpour et al., 2012; Şahin et al., 2024)
Fa2N-4	+ Consistent and reproducible + Express ADME genes like PHH + Higher mRNA level of MDR1	– Low basal level of CAR nuclear receptor and some enzymes such as CYP1A2, CYP1A1, CYP2D6, CYP2E1, UGT1A1, UGT1A6 etc – Incapable of metabolizing compounds	• Cocktail approach • Pathway-Specific Toxicological Studies	(Mills et al., 2004; Ramboer et al., 2015; Youdim et al., 2007)
HepG2	+ Easy handling and low cost + Stable growth + Active phase II metabolism enzymes + High antioxidant capacity + Metabolic specificity	– Low CYPs expression compared to PHH – Tumour-Derived Phenotype – Altered lipid metabolism – Interlaboratory variability	• Cytotoxic assays • Pathway-Specific Toxicological Studies • ROS formation assays • Transcriptomic profiling • Mitochondrial toxicity screening • Genotoxicity prediction (combined with Ames's test) • Phospholipidosis screening	(Donato et al., 2008; Jennen et al., 2010; Kamalian et al., 2015; Magkoufopoulou et al., 2012; Meex et al., 2011; Mišík et al., 2019; Ramirez et al., 2018; Sawada et al., 2005; Schoonen et al., 2005; Stanley & Wolf, 2022; Waldherr et al., 2018)
HuH-7	+ High susceptibility to hepatitis C virus + Higher ability to secrete lipoproteins + Capable of bioactivating acetaminophen + Significant activity of the multidrug-resistant protein	– Tumor-Derived Phenotype – Genomic Instability – Altered CYPs expression compared to PHH	• Drug Transport and Multidrug Resistance Studies • studying HCV biology	(Jouan et al., 2016; Lohmann et al., 1999; Malinen et al., 2019; Marroquin et al. 2007; Nelson et al., 2015; Sawada et al., 2005)

Continuation of the table

Models	Advantages	Disadvantages	Application	References
HepaRG	+ High similarity to PHH + High expression and inducibility of CYPs + Functional Phase II Enzymes and Transporters + Mixed population of hepatocytes and cholangiocytes	– Complex and time-consuming differentiation – Higher Cost in comparison to HepG2 – Less sensitive in detecting some direct- and indirect genotoxic carcinogens	• Hepatotoxicity • DILI prediction • Metabolism and biotransformation of xenobiotics • Genotoxicity prediction • Oxidative stress assays • Detection of drug-induced mitochondrial toxicity	(Aninat et al., 2006; Cerec et al., 2007; Guo et al., 2020; Hart et al., 2010; Mandon et al., 2019; Mišík et al., 2019; Seo et al., 2019, 2020; Tascher et al., 2019; Zheng et al., 2021)

3D cultures

The 3D cellular model combines the advantages of conventional 2D cultivation models, such as lower labour intensity, fever ethically debates compared to live animal models, which makes 3D culture more powerful and widely used *in vitro* model. Currently, 3D models are widely used in fields such as biology, medicine, and pharmacy (Brancato et al., 2020). In addition, 3D culture can also provide a good cellular microenvironment that resembles the real internal conditions of the human body (Fig.2), and thus respond more realistically to environmental pollution (S. Yang et al., 2021; H. Wang et al., 2023).

Spherical hepatic culture. As mentioned before, PHH in 2D monolayer culture rapidly de-differentiate and lose hepatocyte-specific functions. To overcome this issue PHH can be cultured as 3D spheroids. It was shown that this 3D culture can be maintained for longer time in comparison to 2D PHH and produce albumin and urea. It was found that in comparison to 2D PHH the level of expression of proteins involving in drug ADME and catalytic activities of CYPs enzymes, were significantly higher in 3D spheroid cultures (Bell et al., 2018). Moreover, they can form a functional network of bile channels extending from the surface to the inside of the spheroids after 3-4 weeks of cultivation (Tostões et al., 2012). PHH spheroids can also be obtained using bioreactors (Mueller et al., 2011). However, there are some limitations connected to 3D PHH spheroids such as inability to control spheroids` size, difficulty in suspension of them in medium and its replacement.

Except PHH spheroids nowadays several 3D HepaRG models were obtained, which can mimic *in vivo* like microenvironments. In comparison to 2D HepaRG they show improved differentiation, functionality and longevity (Darnell et al., 2012; Gunness et al., 2013; Leite et al., 2012). There are as well HepG2 spheroids that actively used as biosensor-like cell based system to evaluate genotoxicity (Štampar et al., 2022). Compared to 2D HepG2 cells, 3D HepG2 spheroids showed time-dependent decrease in cell proliferation, increased liver function specificity and demonstrated strong physiological relevance in terms of the expression of liver marker genes and metabolic enzymes (Štampar et al., 2020).

3D hepatic organoids. Organoids are 3D self-organised cells derived mostly from stem cells that mimic tissues and organs by recreating their basic functions on a small scale through cell-cell and cell-matrix interaction (Lancaster & Knoblich, 2014). They allow modelling human diseases to evaluate therapeutic strategies. For instance, Ramli *et al.* developed liver organoid with gene expression signature of nonalcoholic steatohepatitis, destruction of the bile duct network and ductal reactions (Ramli et al., 2020). Another model is CFTR-mutant cholangiocyte organoids, which were used to study the patient-specific response to CFTR modulators in patients with cystic fibrosis (Sampaziotis et al., 2021). De Crignis *et al.* generated transgenic organoids to model hepatitis B and C virus infections, which can enables drug screening and identification of cancer-related gene signatures in hepatitis B-infected tissues obtained from patients (De Crignis et al., 2021).

Organoids are also widely used in the assessment of drug toxicity, drug development, and pharmacological research. For example, Bronsard *et al.* obtained 3D multi-cell-type liver organoids using different cell cultures such as primary human macrophages, HepaRG, and hepatic-stellate-cell-derived LX-2 cells to model drug-induced liver injury (Bronsard et al., 2024). Another example is the human liver organoid model developed by Wu *et al.* which has successfully model liver fibrogenesis after stimulation of TGF-β or LPS and has demonstrated application in screening of antifibrotic drugs and drug safety testing (Wu et al., 2023).

Extracellular matrix 3D models. The understanding that the interaction of cells with the extracellular matrix (ECM) affects the morphology, function of cells, as well as their therapeutic response has influenced the development of other types of 3D models that are embedded in the matrix. For example, researchers from Italy have presented a hybrid alginate and ECM hydrogel for creating 3D *in vitro* models of the liver called Hep3Gel to reproduce the chemical and mechanical behaviour of the liver with the possibility of adapting its shape to various production technologies. The viscoelastic properties of Hep3Gel were adjusted considering the available knowledge about liver biomechanics, which made it possible to reproduce the properties of a physiological organ. In addition, this model can be used as a bioink for 3D printing (Guagliano et al., 2023).

Another example of ECM use a method that makes it possible to isolate and further use the main types of human liver cells from cryopreserved material which was developed by Belgium team (Alevra Sarika et al., 2020). Calitz *et al.* showed that composition of ECM can influence on HCC behaviour and increase liver stiffness (Calitz et al., 2023). There are some studies which applied hepatic spheroids of PHH or HepaRG cells into collogen I ECM (Rose et al., 2021, 2022). Liao et al. showed that HepaRG spheroids embedded with collagen accelerate differentiation of cells compared to spheroids grown in suspension (Liao et al., 2022). However, most of the ECM are made from animal origin hydrogels which can create ethical issues and limit its use in human toxicity testing. Another option is synthetic hydrogels, which have reproducible quality, lower immunogenicity, greater mechanical strength, and are easily modifiable. However, they are less biologically active and lack viscoelasticity (Ye et al., 2019). One of the examples of synthetic hydrogen is HepMat gel developed by Kumar *et al.* a fully defined polyethylene glycol-based synthetic hydrogel functionalized to support co-culture of pluripotent stem cell derived hepatocyte- and non-parenchymal cellular -like cells. This system can model the key molecular and functional features of fatty acid-induced inflammation, TGFβ-induced liver fibrosis better than monocultures (Kumar et al., 2021).

The other method that can be related to ECM is collagen-based sandwich culture, where PHH cultured between two layers of collogen gel. Sandwich culture is better replicates the *in vivo* liver microenvironment and preserves key liver functions, including albumin and urea secretion, CYP activity, liver polarity, and bile duct formation in comparison to 2D cells (Dunn et al., 1989; Keemink et al., 2015). Consequently, this system is widely used for studies of drug clearance and polarity-dependent processes such as endocytosis (Treijtel et al., 2004; Zeigerer et al., 2017). Despite these advantages, sandwich cultures prone to cholestasis due to changes in bile metabolism, which limits their long-term preservation (Swift et al., 2010).

3D bioprinting. Currently, 3D printing is a widely popular field and has expanded its applications in regenerative medicine, tissue engineering, and drug screening. This technology allows to construct complex 3D structures which contains multiple cell types and biomaterials. Using this technology researcher can control of the mechanical and biological characteristics of 3D structures with high resolution along three dimensions. Using special bio-inks, it is possible to precisely tune the response or activity of cells to mimic the *in vitro* environment (Ma et al., 2018). There are various methods for 3D bioprinting such as extrusion-based bioprinting, inkjet-based bioprinting, acoustic bioprinting, laser-based bioprinting, at photopolymerization bioprinting (VPB) and magnetic bioprinting (Matai et al., 2020; Shukla et al., 2022)

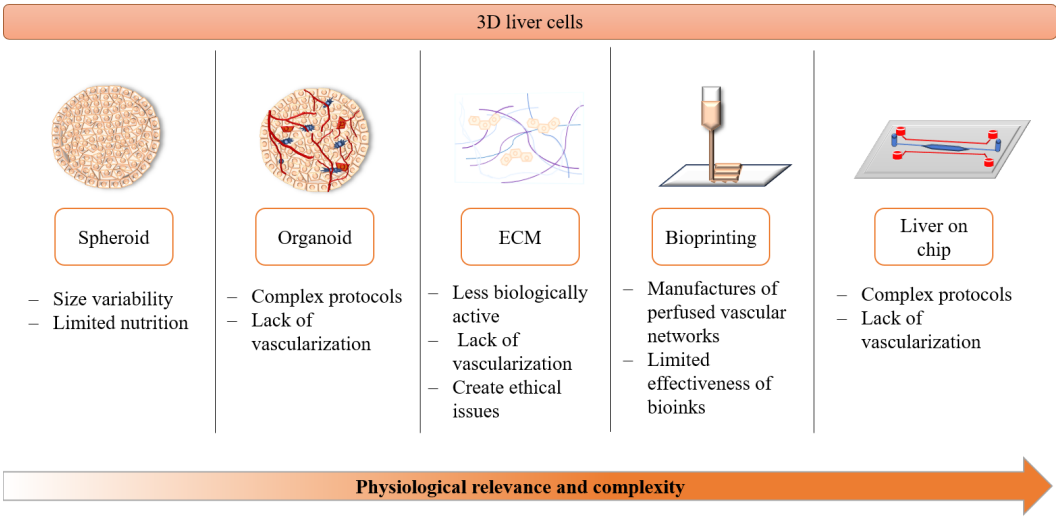
Extrusion-based bioprinting allows the printing of the main components of liver units, while requiring only a simple and easily installed experimental setup. For applications involving microvessels, high-resolution DLP technology can be used to produce more complex matrices with excellent resolution. For 3D liver bioprinting bioinks contains hydrogels and different cells such as hepatic parenchymal cells (hepatocytes) and liver nonparenchymal cells (hepatic stellate cells, hepatic sinusoidal endothelial cells, and Kupffer cells) (W. Li et al., 2023). This technology was already used to mimic different diseases such as hepatocellular carcinoma (Xie et al., 2021) and fibrosis (van Grunsven, 2017). 3D bioprinter liver also can be applied in organ regeneration (Y. Yang et al., 2023) and drug screening (J.-Z. Wang et al., 2018). One of the latest bioprinted liver tissue on the basis of functional hepatocyte organoids derived from human chemically induced pluripotent stem cells was constructed by Li *et al.*, 2025. They showed that this model significantly reduced liver damage, inflammation, and fibrosis, while promoting liver regeneration and expression of biological functions in mice with acute chronic liver failure caused by CCl_4, as well as *Fah* -/- mice (G. Li et al., 2025). Another recently developed method is a 3D model of the human liver made of a disc-shaped structure from a mixture of PHH cells and collagen-1 placed in a highly permeable support medium made of polyethylene glycol

(PEG) microgels. This method has proven to be accurate, precise, and scalable. One hundred tissues can be produced per hour with a variability and error in diameter of about 4%. Histological and immunohistochemical evaluation showed self-organisation, cell cohesion, and expression of key markers in the liver (Subramaniam et al., 2025). Bioprinting represents a promising approach for use in liver toxicity testing, drug development, and organ regeneration. However, a number of challenges remain, including the manufacture of perfused vascular networks that play an important role in liver function, the accurate reproduction of the complex liver microenvironment, and the limited effectiveness of currently available bioinks (W. Li et al., 2023).

Organ-on-chip. The liver on a chip recreates the real conditions of liver functioning in an *in vitro* environment, such as a constant flow of blood and bile. With the help of this technology, a continuous flow of nutrient medium passes through microchannels, which delivers nutrients, oxygen and removes metabolic products (Ehrlich et al., 2019). Thanks to this flow, the liver cells on the chip retain their specialization better, metabolize drugs more actively, synthesize albumin and urea, and react correctly to toxic substances.

Liver in chip mostly used in disease modelling. For example, Gori *et al.* presented liver-on-chip *in vitro* models of human Non-alcoholic fatty liver disease, which enables higher hepatic cell viability, minimal oxidative stress and gradual and lower intracellular lipid accumulation (Gori et al., 2016). Hou *et al.* developed integrated Biomimetic Array Chip which is co-culture model that simultaneously evaluates anti-cancer efficacy and liver toxicity by integrating 3D models of liver and tumour tissues, which improves the accuracy of drug screening at early stages (Hou et al., 2020). Another study tested if liver-in-chip reliably detects toxicity of known hepatotoxin Diglycolic acid. They showed that this method allows reliable detect the hepatotoxicity with high sensitivity, low variability and improved physiological significance compared to traditional PHH multiwell plate format, which confirms its potential use in the regulatory assessment of drug safety (Eckstrum et al., 2020). Recent study also demonstrated the use of liver-on-chip for liver metabolism study and for genotoxicity assay to detect both direct and metabolically activated DNA damage, providing a promising, animal-free tool for early drug safety testing (Kopp et al., 2024). It was even shown that liver on-chip can model in some extend gut-liver interaction. This study demonstrated a multiorgan-on-a-chip platform that combines the human microbial-crosstalk gut-on-chip and the Dynamic42 liver-on-chip platform. This platform captures the influence of gut microbes on human drug metabolism, exemplified by E. coli converting irinotecan metabolites back into toxic forms. This is offering a human-relevant tool for studying microbiome-drug interactions and improving drug safety prediction (Lucchetti et al., 2024). Despite their advantages, liver-on-a-chip models still face several limitations. Their manufacture is a time-consuming and expensive process, and the accuracy and reproducibility of chips largely depend on the choice of materials and methods used in their creation (Liu et al., 2024).

Figure 2
3D culture methods and their disadvantages (ECM – extracellular matrix)



Conclusion

This review compiles and analyses information on modern liver models used in toxicological research. Currently, human liver models are being increasingly refined and various approaches are being explored, including the selection of different cell types and advanced culture techniques, with the aim of more accurately modelling the conditions of liver function *in vivo* to replace animal liver models.

Both 2D and 3D liver models play critical role in toxicology studies. Traditional 2D cultures remain widely used due to their simplicity, cost-effectiveness, reproducibility. They provide valuable insights into basic cellular mechanisms, toxicity testing and early stage of drug development. However, their inability to accurately reproduce the complex processes of native liver tissues limit their physiological relevance.

In contrast, modern *in vitro* liver models have significantly advanced toxicological studies by providing more physiologically relevant systems than early 2D hepatocyte cultures. The 3D cell models combine the advantages of conventional 2D cultivation models, such as lower labour intensity and the absence of ethical issues compared to live animal models, which makes the 3D culture a more powerful and widely used *in vitro* model. The use of various cell types and sophisticated cultivation methods has improved the prediction of the compounds' effects on the liver. However, problems such as phenotypic drift during long-term cultivation, limited suitability, lack of vascularization, etc., still limit their full-fledged use as an alternative to animal models. Despite these limitations, the continuous improvement of these models promises to provide more accurate tools for studying the occurrence, progression, and potential reversibility of hepatotoxicity. This will contribute to a safer and more effective assessment of chemicals and drugs in preclinical toxicology.

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Conflict of interest

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References

1. Alevra Sarika, N., Payen, V. L., Fléron, M., Ravau, J., Brusa, D., Najimi, M., De Pauw, E., Eppe, G., Mazzucchelli, G., Sokal, E. M., des Rieux, A., & El Taghdouini, A. (2020). Human Liver-Derived Extracellular Matrix for the Culture of Distinct Human Primary Liver Cells. *Cells, 9*(6), 1357. https://doi.org/10.3390/cells9061357

2. Aninat, C., Piton, A., Glaise, D., Le Charpentier, T., Langouët, S., Morel, F., Guguen-Guillouzo, C., & Guillouzo, A. (2006). Expression of cytochromes P450, conjugating enzymes and nuclear receptors in human hepatoma HepaRG cells. *Drug Metabolism and Disposition, 34*(1), 75–83. https://doi.org/10.1124/dmd.105.006759

3. Baratzhanova, G., El Sheikh Saad, H., Fournier, A., Huguet, M., Joubert, O., Paul, A., Djansugurova, L., & Cakir-Kiefer, C. (2025). Comparison of the impact of chlordecone and its metabolite chlordecol on genes involved in pesticide metabolism in HepG2 cell line. *Environmental Toxicology and Pharmacology, 116,* 104701. https://doi.org/10.1016/j.etap.2025.104701

4. Bell, C. C., Dankers, A. C. A., Lauschke, V. M., Sison-Young, R., Jenkins, R., Rowe, C., Goldring, C. E., Park, K., Regan, S. L., Walker, T., Schofield, C., Baze, A., Foster, A. J., Williams, D. P., van de Ven, A. W. M., Jacobs, F., van Houdt, J., Lähteenmäki, T., Snoeys, J., … Ingelman-Sundberg, M. (2018). Comparison of Hepatic 2D Sandwich Cultures and 3D Spheroids for Long-term Toxicity Applications: A Multicenter Study. *Toxicological Sciences, 162*(2), 655–666. https://doi.org/10.1093/toxsci/kfx289

5. Berger, B., Donzelli, M., Maseneni, S., Boess, F., Roth, A., Krähenbühl, S., & Haschke, M. (2016). Comparison of Liver Cell Models Using the Basel Phenotyping Cocktail. *Frontiers in Pharmacology, 7*. https://doi.org/10.3389/fphar.2016.00443

6. Brancato, V., Oliveira, J. M., Correlo, V. M., Reis, R. L., & Kundu, S. C. (2020). Could 3D models of cancer enhance drug screening? *Biomaterials, 232*, 119744. https://doi.org/10.1016/j.biomaterials.2019.119744

7. Bronsard, J., Savary, C., Massart, J., Viel, R., Moutaux, L., Catheline, D., Rioux, V., Clement, B., Corlu, A., Fromenty, B., & Ferron, P. J. (2024). 3D multi-cell-type liver organoids: A new model of non-alcoholic fatty liver disease for drug safety assessments. *Toxicology in Vitro: An International Journal Published in Association with BIBRA, 94*, 105728. https://doi.org/10.1016/j.tiv.2023.105728

8. Calitz, C., Rosenquist, J., Degerstedt, O., Khaled, J., Kopsida, M., Fryknäs, M., Lennernäs, H., Samanta, A., & Heindryckx, F. (2023). Influence of extracellular matrix composition on tumour cell behaviour in a biomimetic in vitro model for hepatocellular carcinoma. *Scientific Reports, 13*(1), 748. https://doi.org/10.1038/s41598-023-27997-3

9. Castell, J. V., Jover, R., Martnez-Jimnez, C. P., & Gmez-Lechn, M. J. (2006). Hepatocyte cell lines: Their use, scope and limitations in drug metabolism studies. *Expert Opinion on Drug Metabolism & Toxicology, 2*(2), 183–212. https://doi.org/10.1517/17425255.2.2.183

10. Cerec, V., Glaise, D., Garnier, D., Morosan, S., Turlin, B., Drenou, B., Gripon, P., Kremsdorf, D., Guguen-Guillouzo, C., & Corlu, A. (2007). Transdifferentiation of hepatocyte-like cells from the human hepatoma HepaRG cell line through bipotent progenitor. *Hepatology, 45*(4), 957–967. https://doi.org/10.1002/hep.21536

11. Dao Thi, V. L., Wu, X., Belote, R. L., Andreo, U., Takacs, C. N., Fernandez, J. P., Vale-Silva, L. A., Prallet, S., Decker, C. C., Fu, R. M., Qu, B., Uryu, K., Molina, H., Saeed, M., Steinmann, E., Urban, S., Singaraja, R. R., Schneider, W. M., Simon, S. M., & Rice, C. M. (2020). Stem cell-derived polarized hepatocytes. N*ature Communications, 11*(1), 1677. https://doi.org/10.1038/s41467-020-15337-2

12. Darnell, M., Ulvestad, M., Ellis, E., Weidolf, L., & Andersson, T. B. (2012). In vitro evaluation of major in vivo drug metabolic pathways using primary human hepatocytes and HepaRG cells in suspension and a dynamic three-dimensional bioreactor system. *The Journal of Pharmacology and Experimental Therapeutics, 343*(1), 134–144. https://doi.org/10.1124/jpet.112.195834

13. De Crignis, E., Hossain, T., Romal, S., Carofiglio, F., Moulos, P., Khalid, M. M., Rao, S., Bazrafshan, A., Verstegen, M. M., Pourfarzad, F., Koutsothanassis, C., Gehart, H., Kan, T. W., Palstra, R.-J., Boucher, C., IJzermans, J. N., Huch, M., Boj, S. F., Vries, R., … Mahmoudi, T. (2021). Application of human liver organoids as a patient-derived primary model for HBV infection and related hepatocellular carcinoma. *eLife, 10*, e60747. https://doi.org/10.7554/eLife.60747

14. Dehn, P. F., Allen-Mocherie, S., Karek, J., & Thenappan, A. (2005). Organochlorine insecticides: Impacts on human HepG2 cytochrome P4501A, 2B activities and glutathione levels. *Toxicology in Vitro, 19*(2), 261–273. https://doi.org/10.1016/j.tiv.2004.10.002

15. Donato, M. T., Jover, R., & Gómez-Lechón, M. J. (2013). Hepatic cell lines for drug hepatotoxicity testing: Limitations and strategies to upgrade their metabolic competence by gene engineering. *Current Drug Metabolism, 14*(9), 946–968. https://doi.org/10.2174/1389200211314090002

16. Donato, M. T., Lahoz, A., Castell, J. V., & Gómez-Lechón, M. J. (2008). Cell lines: A tool for in vitro drug metabolism studies. *Current Drug Metabolism, 9*(1), 1–11. https://doi.org/10.2174/138920008783331086

17. Donowitz, M., Turner, J. R., Verkman, A. S., & Zachos, N. C. (2020). Current and potential future applications of human stem cell models in drug development. *The Journal of Clinical Investigation, 130*(7), 3342–3344. https://doi.org/10.1172/JCI138645

18. Dorr, C. R., Remmel, R. P., Muthusamy, A., Fisher, J., Moriarity, B. S., Yasuda, K., Wu, B., Guan, W., Schuetz, E. G., Oetting, W. S., Jacobson, P. A., & Israni, A. K. (2017). CRISPR/Cas9 Genetic Modification of CYP3A5 *3 in HuH-7 Human Hepatocyte Cell Line Leads to Cell Lines with Increased Midazolam and Tacrolimus Metabolism. *Drug Metabolism and Disposition, 45*(8), 957–965. https://doi.org/10.1124/dmd.117.076307

19. Dunn, J. C., Yarmush, M. L., Koebe, H. G., & Tompkins, R. G. (1989). Hepatocyte function and extracellular matrix geometry: Long-term culture in a sandwich configuration. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 3*(2), 174–177. https://doi.org/10.1096/fasebj.3.2.2914628

20. Eckstrum, K., Striz, A., Ferguson, M., Zhao, Y., Welch, B., Solomotis, N., Olejnik, N., & Sprando, R. (2020). Utilization of a model hepatotoxic compound, diglycolic acid, to evaluate liver Organ-Chip performance and in vitro to in vivo concordance. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 146*, 111850. https://doi.org/10.1016/j.fct.2020.111850

21. Ehrlich, A., Duche, D., Ouedraogo, G., & Nahmias, Y. (2019). Challenges and Opportunities in the Design of Liver-on-Chip Microdevices. *An. Rev. of Biomed. Engineering, 21*, 219–239. https://doi.org/10.1146/annurev-bioeng-060418-052305

22. Fukunaga, I., & Takebe, T. (2025). In vitro liver models for toxicological research. Drug Metabolism and *Pharmacokinetics, 62*, 101478. https://doi.org/10.1016/j.dmpk.2025.101478

23. Godoy, P., Hewitt, N. J., Albrecht, U., Andersen, M. E., Ansari, N., Bhattacharya, S., Bode, J. G., Bolleyn, J., Borner, C., Böttger, J., Braeuning, A., Budinsky, R. A., Burkhardt, B., Cameron, N. R., Camussi, G., Cho, C.-S., Choi, Y.-J., Craig Rowlands, J., Dahmen, U., … Hengstler, J. G. (2013). 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. *Archives of Toxicology, 87*(8), 1315–1530. https://doi.org/10.1007/s00204-013-1078-5

24. Gómez-Lechón, M. J., & Tolosa, L. (2016). Human hepatocytes derived from pluripotent stem cells: A promising cell model for drug hepatotoxicity screening. *Archives of Toxicology, 90*(9), 2049–2061. https://doi.org/10.1007/s00204-016-1756-1

25. Gori, M., Simonelli, M. C., Giannitelli, S. M., Businaro, L., Trombetta, M., & Rainer, A. (2016). Investigating Nonalcoholic Fatty Liver Disease in a Liver-on-a-Chip Microfluidic Device. *PloS One, 11*(7), e0159729. https://doi.org/10.1371/journal.pone.0159729

26. Gracey, E. G., & Lampe, J. N. (2025). Novel emerging cell and organoid systems for the study of drug metabolism and toxicity in humans. *Drug Metabolism and Disposition, 53*(12), 100188. https://doi.org/10.1016/j.dmd.2025.100188

27. Graffmann, N., Scherer, B., & Adjaye, J. (2022). In vitro differentiation of pluripotent stem cells into hepatocyte like cells – Basic principles and current progress. *Stem Cell Research, 61*, 102763. https://doi.org/10.1016/j.scr.2022.102763

28. Guagliano, G., Volpini, C., Sardelli, L., Bloise, N., Briatico-Vangosa, F., Cornaglia, A. I., Dotti, S., Villa, R., Visai, L., & Petrini, P. (2023). Hep3Gel: A Shape-Shifting Extracellular Matrix-Based, Three-Dimensional Liver Model Adaptable to Different Culture Systems. *ACS Biomaterials Science & Engineering, 9*(1), 211–229. https://doi.org/10.1021/acsbiomaterials.2c01226

29. Guengerich, F. P. (2011). Mechanisms of Drug Toxicity and Relevance to Pharmaceutical Development. *Drug Metabolism and Pharmacokinetics, 26*(1), 3–14. https://doi.org/10.2133/dmpk.dmpk-10-rv-062

30. Gunness, P., Mueller, D., 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: An Official Journal of the Society of Toxicology, 133*(1), 67–78. https://doi.org/10.1093/toxsci/kft021

31. Guo, X., Seo, J.-E., Petibone, D., Tryndyak, V., Lee, U. J., Zhou, T., Robison, T. W., & Mei, N. (2020). Performance of HepaRG and HepG2 cells in the high-throughput micronucleus assay for in vitro genotoxicity assessment. *Journal of Toxicology and Environmental Health, Part A, 83*(21–22), 702–717. https://doi.org/10.1080/15287394.2020.1822972

32. Hariparsad, N., Carr, B. A., Evers, R., & Chu, X. (2008). Comparison of immortalized Fa2N-4 cells and human hepatocytes as in vitro models for cytochrome P450 induction. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 36*(6), 1046–1055. https://doi.org/10.1124/dmd.108.020677

33. Hart, S. N., Li, Y., Nakamoto, K., Subileau, E., Steen, D., & Zhong, X. (2010). A Comparison of Whole Genome Gene Expression Profiles of HepaRG Cells and HepG2 Cells to Primary Human Hepatocytes and Human Liver Tissues. *Drug Metabolism and Disposition, 38*(6), 988–994. https://doi.org/10.1124/dmd.109.031831

34. Hou, Y., Ai, X., Zhao, L., Gao, Z., Wang, Y., Lu, Y., Tu, P., & Jiang, Y. (2020). An integrated biomimetic array chip for high-throughput co-culture of liver and tumor microtissues for advanced anticancer bioactivity screening. *Lab on a Chip, 20*(14), 2482–2494. https://doi.org/10.1039/d0lc00288g

35. Jennen, D. G. J., Magkoufopoulou, C., Ketelslegers, H. B., van Herwijnen, M. H. M., Kleinjans, J. C. S., & van Delft, J. H. M. (2010). Comparison of HepG2 and HepaRG by whole-genome gene expression analysis for the purpose of chemical hazard identification. *Toxicological Sciences: An Official Journal of the Society of Toxicology*, 115(1), 66–79. https://doi.org/10.1093/toxsci/kfq026

36. Jouan, E., Vée, M. L., Denizot, C., Parmentier, Y., & Fardel, O. (2016). Drug Transporter Expression and Activity in Human Hepatoma HuH-7 Cells. *Pharmaceutics, 9*(1). https://doi.org/10.3390/pharmaceutics9010003

37. Kamalian, L., Chadwick, A. E., Bayliss, M., French, N. S., Monshouwer, M., Snoeys, J., & Park, B. K. (2015). The utility of HepG2 cells to identify direct mitochondrial dysfunction in the absence of cell death. *Toxicology in Vitro, 29*(4), 732–740. https://doi.org/10.1016/j.tiv.2015.02.011

38. Kammerer, S., & Küpper, J.-H. (2018). Human hepatocyte systems for in vitro toxicology analysis. *Journal of Cellular Biotechnology, 3*(2), 85–93. https://doi.org/10.3233/JCB-179012

39. Kasai, F., Hirayama, N., Ozawa, M., Satoh, M., & Kohara, A. (2018). HuH-7 reference genome profile: Complex karyotype composed of massive loss of heterozygosity. *Human Cell, 31*(3), 261–267. https://doi.org/10.1007/s13577-018-0212-3

40. Keemink, J., Oorts, M., & Annaert, P. (2015). Primary Hepatocytes in Sandwich Culture. Methods in *Molecular Biology, 1250*, 175–188. https://doi.org/10.1007/978-1-4939-2074-7_12

41. Knasmüller, S., Mersch-Sundermann, V., Kevekordes, S., Darroudi, F., Huber, W. W., Hoelzl, C., Bichler, J., & Majer, B. J. (2004). Use of human-derived liver cell lines for the detection of environmental and dietary genotoxicants; current state of knowledge. *Toxicology, 198*(1–3), 315–328. https://doi.org/10.1016/j.tox.2004.02.008

42. Kopp, B., Khawam, A., Di Perna, K., Lenart, D., Vinette, M., Silva, R., Zanoni, T. B., Rore, C., Guenigault, G., Richardson, E., Kostrzewski, T., Boswell, A., Van, P., Valentine Iii, C., Salk, J., & Hamel, A. (2024). Liver-on-chip model and application in predictive genotoxicity and mutagenicity of drugs. Mutation Research. *Genetic Toxicology and Environmental Mutagenesis, 896*, 503762. https://doi.org/10.1016/j.mrgentox.2024.503762

43. Kumar, M., Toprakhisar, B., Van Haele, M., Antoranz, A., Boon, R., Chesnais, F., De Smedt, J., Tricot, T., Idoype, T. I., Canella, M., Tilliole, P., De Boeck, J., Bajaj, M., Ranga, A., Bosisio, F. M., Roskams, T., van Grunsven, L. A., & Verfaillie, C. M. (2021). A fully defined matrix to support a pluripotent stem cell derived multi-cell-liver steatohepatitis and fibrosis model. *Biomaterials, 276*, 121006. https://doi.org/10.1016/j.biomaterials.2021.121006

44. Lancaster, M. A., & Knoblich, J. A. (2014). Organogenesis in a dish: Modeling development and disease using organoid technologies. *Science, 345*(6194), 1247125. https://doi.org/10.1126/science.1247125

45. Leite, S. B., Wilk-Zasadna, I., Zaldivar, J. M., Airola, E., Reis-Fernandes, M. A., Mennecozzi, M., Guguen-Guillouzo, C., Chesne, C., Guillou, C., Alves, P. M., & Coecke, S. (2012). Three-dimensional HepaRG model as an attractive tool for toxicity testing. T*oxicological Sciences: An Official Journal of the Society of Toxicology, 130*(1), 106–116. https://doi.org/10.1093/toxsci/kfs232

46. Li, G., He, J., Shi, J., Li, X., Liu, L., Ge, X., Chen, W., Jia, J., Wang, J., Yin, M., Sakai, Y., Sun, W., Deng, H., & Pang, Y. (2025). Bioprinting functional hepatocyte organoids derived from human chemically induced pluripotent stem cells to treat liver failure. *Gut, 74*(7), 1150–1164. https://doi.org/10.1136/gutjnl-2024-333885

47. Li, W., Liu, Z., Tang, F., Jiang, H., Zhou, Z., Hao, X., & Zhang, J. M. (2023). Application of 3D Bioprinting in Liver Diseases. *Micromachines, 14*(8), 1648. https://doi.org/10.3390/mi14081648

48. Liao, F.-C., Wang, Y.-K., Cheng, M.-Y., & Tu, T.-Y. (2022). A Preliminary Investigation of Embedding In Vitro HepaRG Spheroids into Recombinant Human Collagen Type I for the Promotion of Liver Differentiation. *Polymers, 14*(9). https://doi.org/10.3390/polym14091923

49. Liguori, M. J., Blomme, E. A. G., & Waring, J. F. (2008). Trovafloxacin-induced gene expression changes in liver-derived in vitro systems: Comparison of primary human hepatocytes to HepG2 cells. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 36*(2), 223–233. https://doi.org/10.1124/dmd.107.017608

50. Liu, J., Du, Y., Xiao, X., Tan, D., He, Y., & Qin, L. (2024). Construction of in vitro liver-on-a-chip models and application progress. *BioMedical Engineering OnLine, 23*(1), 33. https://doi.org/10.1186/s12938-024-01226-y

51. Lohmann, V., Körner, F., Koch, J., Herian, U., Theilmann, L., & Bartenschlager, R. (1999). Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science (New York, N.Y.), 285*(5424), 110–113. https://doi.org/10.1126/science.285.5424.110

52. Lübberstedt, M., Müller-Vieira, U., Mayer, M., Biemel, K. M., Knöspel, F., Knobeloch, D., Nüssler, A. K., Gerlach, J. C., & Zeilinger, K. (2011). HepaRG human hepatic cell line utility as a surrogate for primary human hepatocytes in drug metabolism assessment in vitro. *Journal of Pharmacological and Toxicological Methods, 63*(1), 59–68. https://doi.org/10.1016/j.vascn.2010.04.013

53. Lucchetti, M., Aina, K. O., Grandmougin, L., Jäger, C., Pérez Escriva, P., Letellier, E., Mosig, A. S., & Wilmes, P. (2024). An Organ-on-Chip Platform for Simulating Drug Metabolism Along the Gut-Liver Axis. *Advanced Healthcare Materials, 13*(20), e2303943. https://doi.org/10.1002/adhm.202303943

54. Ma, X., Liu, J., Zhu, W., Tang, M., Lawrence, N., Yu, C., Gou, M., & Chen, S. (2018). 3D bioprinting of functional tissue models for personalized drug screening and in vitro disease modeling. *Advanced Drug Delivery Reviews, 132*, 235–251. https://doi.org/10.1016/j.addr.2018.06.011

55. Magkoufopoulou, C., Claessen, S. M. H., Tsamou, M., Jennen, D. G. J., Kleinjans, J. C. S., & van Delft, J. H. M. (2012). A transcriptomics-based in vitro assay for predicting chemical genotoxicity in vivo. *Carcinogenesis, 33*(7), 1421–1429. https://doi.org/10.1093/carcin/bgs182

56. Malinen, M. M., Ito, K., Kang, H. E., Honkakoski, P., & Brouwer, K. L. R. (2019). Protein expression and function of organic anion transporters in short-term and long-term cultures of Huh7 human hepatoma cells. *European Journal of Pharmaceutical Sciences, 130*, 186–195. https://doi.org/10.1016/j.ejps.2019.01.022

57. Mandon, M., Huet, S., Dubreil, E., Fessard, V., & Le Hégarat, L. (2019). Three-dimensional HepaRG spheroids as a liver model to study human genotoxicity in vitro with the single cell gel electrophoresis assay. *Scientific Reports, 9*(1), Article 1. https://doi.org/10.1038/s41598-019-47114-7

58. Mann, D. A. (2015). Human induced pluripotent stem cell-derived hepatocytes for toxicology testing. *Expert Opinion on Drug Metabolism & Toxicology, 11*(1), 1–5. https://doi.org/10.1517/17425255.2015.981523

59. Marion, M.-J., Hantz, O., & Durantel, D. (2010). The HepaRG Cell Line: Biological Properties and Relevance as a Tool for Cell Biology, Drug Metabolism, and Virology Studies. In P. Maurel (Ed.), Hepatocytes (Vol. 640, pp. 261–272). Humana Press. https://doi.org/10.1007/978-1-60761-688-7_13

60. Marroquin, L. D., Hynes, J., Dykens, J. A., Jamieson, J. D., & Will, Y. (2007). Circumventing the Crabtree Effect: Replacing Media Glucose with Galactose Increases Susceptibility of HepG2 Cells to Mitochondrial Toxicants. *Toxicological Sciences, 97*(2), 539–547. https://doi.org/10.1093/toxsci/kfm052

61. Matai, I., Kaur, G., Seyedsalehi, A., McClinton, A., & Laurencin, C. T. (2020). Progress in 3D bioprinting technology for tissue/organ regenerative engineering. *Biomaterials, 226*, 119536. https://doi.org/10.1016/j.biomaterials.2019.119536

62. Matsui, T., Miyamoto, N., Saito, F., & Shinozawa, T. (2020). Molecular Profiling of Human Induced Pluripotent Stem Cell-Derived Cells and their Application for Drug Safety Study. *Current Pharmaceutical Biotechnology, 21*(9), 807–828. https://doi.org/10.2174/1389201021666200422090952

63. Meex, S. J. R., Andreo, U., Sparks, J. D., & Fisher, E. A. (2011). Huh-7 or HepG2 cells: Which is the better model for studying human apolipoprotein-B100 assembly and secretion?. *Journal of Lipid Research, 52*(1), 152–158. https://doi.org/10.1194/jlr.D008888

64. Mills, J. B., Rose, K. A., Sadagopan, N., Sahi, J., & de Morais, S. M. F. (2004). Induction of Drug Metabolism Enzymes and MDR1 Using a Novel Human Hepatocyte Cell Line. T*he Journal of Pharmacology and Experimental Therapeutics, 309*(1), 303–309. https://doi.org/10.1124/jpet.103.061713

65. Mišík, M., Nersesyan, A., Ropek, N., Huber, W. W., Haslinger, E., & Knasmueller, S. (2019). Use of human derived liver cells for the detection of genotoxins in comet assays. Mutation Research. *Genetic Toxicology and Environmental Mutagenesis, 845*, 402995. https://doi.org/10.1016/j.mrgentox.2018.12.003

66. Mueller, D., Tascher, G., Müller-Vieira, U., Knobeloch, D., Nuessler, A. K., Zeilinger, K., Heinzle, E., & Noor, F. (2011). In-depth physiological characterization of primary human hepatocytes in a 3D hollow-fiber bioreactor. *Journal of Tissue Engineering and Regenerative Medicine, 5*(8), e207–e218. https://doi.org/10.1002/term.418

67. Nakabayashi, H., Taketa, K., Miyano, K., Yamane, T., & Sato, J. (1982). Growth of human hepatoma cells lines with differentiated functions in chemically defined medium. *Cancer Research, 42*(9), 3858–3863.

68. Nelson, L. J., Navarro, M., Treskes, P., Samuel, K., Tura-Ceide, O., Morley, S. D., Hayes, P. C., & Plevris, J. N. (2015). Acetaminophen cytotoxicity is ameliorated in a human liver organotypic co-culture model. *Scientific Reports, 5*(1), 17455. https://doi.org/10.1038/srep17455

69. Parmentier, C., Hendriks, D. F. G., Heyd, B., Bachellier, P., Ingelman-Sundberg, M., & Richert, L. (2018). Inter-individual differences in the susceptibility of primary human hepatocytes towards drug-induced cholestasis are compound and time dependent. *Toxicology Letters, 295*, 187–194. https://doi.org/10.1016/j.toxlet.2018.06.1069

70. Pfeifer, A. M., Cole, K. E., Smoot, D. T., Weston, A., Groopman, J. D., Shields, P. G., Vignaud, J. M., Juillerat, M., Lipsky, M. M., & Trump, B. F. (1993). Simian virus 40 large tumor antigen-immortalized normal human liver epithelial cells express hepatocyte characteristics and metabolize chemical carcinogens. *Proceedings of the National Academy of Sciences, 90*(11), 5123–5127. https://doi.org/10.1073/pnas.90.11.5123

71. Philippeos, C., Hughes, R. D., Dhawan, A., & Mitry, R. R. (2012). Introduction to Cell Culture. In R. R. Mitry & R. D. Hughes (Eds), Human Cell Culture Protocols (Vol. 806, pp. 1–13). Humana Press. https://doi.org/10.1007/978-1-61779-367-7_1

72. Poh, W. T., & Stanslas, J. (2024). The new paradigm in animal testing – "3Rs alternatives". Regulatory *Toxicology and Pharmacology, 153*, 105705. https://doi.org/10.1016/j.yrtph.2024.105705

73. Ramboer, E., Vanhaecke, T., Rogiers, V., & Vinken, M. (2015). Immortalized human hepatic cell lines for in vitro testing and research purposes. Methods in Molecular Biology (Clifton, N.J.), 1250, 53–76. https://doi.org/10.1007/978-1-4939-2074-7_4

74. Ramirez, T., Strigun, A., Verlohner, A., Huener, H.-A., Peter, E., Herold, M., Bordag, N., Mellert, W., Walk, T., Spitzer, M., Jiang, X., Sperber, S., Hofmann, T., Hartung, T., Kamp, H., & van Ravenzwaay, B. (2018). Prediction of liver toxicity and mode of action using metabolomics in vitro in HepG2 cells. *Archives of Toxicology, 92*(2), 893–906. https://doi.org/10.1007/s00204-017-2079-6

75. Ramli, M. N. B., Lim, Y. S., Koe, C. T., Demircioglu, D., Tng, W., Gonzales, K. A. U., Tan, C. P., Szczerbinska, I., Liang, H., Soe, E. L., Lu, Z., Ariyachet, C., Yu, K. M., Koh, S. H., Yaw, L. P., Jumat, N. H. B., Lim, J. S. Y., Wright, G., Shabbir, A., … Chan, Y.-S. (2020). Human Pluripotent Stem Cell-Derived Organoids as Models of Liver Disease. *Gastroenterology, 159*(4), 1471-1486.e12. https://doi.org/10.1053/j.gastro.2020.06.010

76. Richert, L., Alexandre, E., Lloyd, T., Orr, S., Viollon-Abadie, C., Patel, R., Kingston, S., Berry, D., Dennison, A., Heyd, B., Mantion, G., & Jaeck, D. (2004). Tissue collection, transport and isolation procedures required to optimize human hepatocyte isolation from waste liver surgical resections. A multilaboratory study. *Liver International, 24*(4), 371–378. https://doi.org/10.1111/j.1478-3231.2004.0930.x

77. Roe, A. L., Snawder, J. E., Benson, R. W., Roberts, D. W., & Casciano, D. A. (1993). HepG2 cells: An in vitro model for P450-dependent metabolism of acetaminophen. *Biochemical and Biophysical Research Communications, 190*(1), 15–19. https://doi.org/10.1006/bbrc.1993.1003

78. Rose, S., Cuvellier, M., Ezan, F., Carteret, J., Bruyère, A., Legagneux, V., Nesslany, F., Baffet, G., & Langouët, S. (2022). DMSO-free highly differentiated HepaRG spheroids for chronic toxicity, liver functions and genotoxicity studies. *Archives of Toxicology, 96*(1), 243–258. https://doi.org/10.1007/s00204-021-03178-x

79. Rose, S., Ezan, F., Cuvellier, M., Bruyère, A., Legagneux, V., Langouët, S., & Baffet, G. (2021). Generation of proliferating human adult hepatocytes using optimized 3D culture conditions. *Scientific Reports, 11*(1), 515. https://doi.org/10.1038/s41598-020-80019-4

80. Şahin, S., Azarkan, S. Y., & Türksoy, V. A. (2024). Evaluation of the effect of cannabidiol on the THLE-2 liver cell line exposed to lead. *The Science of the Total Environment, 923*, 170901. https://doi.org/10.1016/j.scitotenv.2024.170901

81. Sampaziotis, F., Muraro, D., Tysoe, O. C., Sawiak, S., Beach, T. E., Godfrey, E. M., Upponi, S. S., Brevini, T., Wesley, B. T., Garcia-Bernardo, J., Mahbubani, K., Canu, G., Gieseck, R., Berntsen, N. L., Mulcahy, V. L., Crick, K., Fear, C., Robinson, S., Swift, L., … Vallier, L. (2021). Cholangiocyte organoids can repair bile ducts after transplantation in the human liver. *Science, 371*(6531), 839–846. https://doi.org/10.1126/science.aaz6964

82. Sawada, H., Takami, K., & Asahi, S. (2005). A toxicogenomic approach to drug-induced phospholipidosis: Analysis of its induction mechanism and establishment of a novel in vitro screening system. Toxicological Sciences: An *Official Journal of the Society of Toxicology, 83*(2), 282–292. https://doi.org/10.1093/toxsci/kfh264

83. Schoonen, W. G. E. J., Westerink, W. M. A., de Roos, J. A. D. M., & Débiton, E. (2005). Cytotoxic effects of 100 reference compounds on Hep G2 and HeLa cells and of 60 compounds on ECC-1 and CHO cells. I mechanistic assays on ROS, glutathione depletion and calcein uptake. *Toxicology in Vitro, 19*(4), 505–516. https://doi.org/10.1016/j.tiv.2005.01.003

84. Sefried, S., Häring, H.-U., Weigert, C., & Eckstein, S. S. (2018). Suitability of hepatocyte cell lines HepG2, AML12 and THLE-2 for investigation of insulin signalling and hepatokine gene expression. *Open Biology, 8*(10), 180147. https://doi.org/10.1098/rsob.180147

85. Segeritz, C.-P., & Vallier, L. (2017). Cell Culture. In Basic Science Methods for Clinical Researchers (pp. 151–172). https://doi.org/10.1016/B978-0-12-803077-6.00009-6

86. Seo, J.-E., Tryndyak, V., Wu, Q., Dreval, K., Pogribny, I., Bryant, M., Zhou, T., Robison, T. W., Mei, N., & Guo, X. (2019). Quantitative comparison of in vitro genotoxicity between metabolically competent HepaRG cells and HepG2 cells using the high-throughput high-content CometChip assay. *Archives of Toxicology, 93*(5), 1433–1448. https://doi.org/10.1007/s00204-019-02406-9

87. Seo, J.-E., Wu, Q., Bryant, M., Ren, L., Shi, Q., Robison, T. W., Mei, N., Manjanatha, M. G., & Guo, X. (2020). Performance of high-throughput CometChip assay using primary human hepatocytes: A comparison of DNA damage responses with in vitro human hepatoma cell lines. *Archives of Toxicology, 94*(6), 2207–2224. https://doi.org/10.1007/s00204-020-02736-z

88. Shah, F., Louise-May, S., & Greene, N. (2014). Chemotypes sensitivity and predictivity of in vivo outcomes for cytotoxic assays in THLE and HepG2 cell lines. *Bioorganic & Medicinal Chemistry Letters, 24*(12), 2753–2757. https://doi.org/10.1016/j.bmcl.2014.04.039

89. Shukla, P., Yeleswarapu, S., Heinrich, M. A., Prakash, J., & Pati, F. (2022). Mimicking tumor microenvironment by 3D bioprinting: 3D cancer modeling. *Biofabrication, 14*(3). https://doi.org/10.1088/1758-5090/ac6d11

90. Shulman, M., & Nahmias, Y. (2013). Long-term culture and coculture of primary rat and human hepatocytes. Methods in Molecular Biology (Clifton, N.J.), 945, 287–302. https://doi.org/10.1007/978-1-62703-125-7_17

91. Soltanpour, Y., Hilgendorf, C., Ahlström, M. M., Foster, A. J., Kenna, J. G., Petersen, A., & Ungell, A.-L. (2012). Characterization of THLE-cytochrome P450 (P450) cell lines: Gene expression background and relationship to P450-enzyme activity. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 40*(11), 2054–2058. https://doi.org/10.1124/dmd.112.045815

92. Štampar, M., Breznik, B., Filipič, M., & Žegura, B. (2020). Characterization of In Vitro 3D Cell Model Developed from Human Hepatocellular Carcinoma (HepG2) Cell Line. *Cells, 9*(12), 2557. https://doi.org/10.3390/cells9122557

93. Štampar, M., Žabkar, S., Filipič, M., & Žegura, B. (2022). HepG2 spheroids as a biosensor-like cell-based system for (geno) toxicity assessment. *Chemosphere, 291*, 132805. https://doi.org/10.1016/j.chemosphere.2021.132805

94. Stanley, L. A., & Wolf, C. R. (2022). Through a glass, darkly? HepaRG and HepG2 cells as models of human phase I drug metabolism. *Drug Metabolism Reviews, 54*(1), 46–62. https://doi.org/10.1080/03602532.2022.2039688

95. Subramaniam, V., Abrahan, C., Higgins, B. R., Chisolm, S. J., Sweeney, B., Duraivel, S., Balzano-Nogueira, L., Monjure, T., Wang, C.-Y., Palmer, G. D., & Angelini, T. E. (2025). A functional human liver tissue model: 3D bioprinted co-culture discoids. *Biomaterials Advances, 173,* 214288. https://doi.org/10.1016/j.bioadv.2025.214288

96. Swift, B., Pfeifer, N. D., & Brouwer, K. L. R. (2010). Sandwich-cultured hepatocytes: An in vitro model to evaluate hepatobiliary transporter-based drug interactions and hepatotoxicity. *Drug Metabolism Reviews, 42*(3), 446–471. https://doi.org/10.3109/03602530903491881

97. Tascher, G., Burban, A., Camus, S., Plumel, M., Chanon, S., Le Guevel, R., Shevchenko, V., Van Dorsselaer, A., Lefai, E., Guguen-Guillouzo, C., & Bertile, F. (2019). In-Depth Proteome Analysis Highlights HepaRG Cells as a Versatile Cell System Surrogate for Primary Human Hepatocytes. *Cells, 8*(2), 192. https://doi.org/10.3390/cells8020192

98. Tostões, R. M., Leite, S. B., Serra, M., Jensen, J., Björquist, P., Carrondo, M. J. T., Brito, C., & Alves, P. M. (2012). Human liver cell spheroids in extended perfusion bioreactor culture for repeated-dose drug testing. *Hepatology (Baltimore, Md.), 55*(4), 1227–1236. https://doi.org/10.1002/hep.24760

99. Treijtel, N., Barendregt, A., Freidig, A. P., Blaauboer, B. J., & van Eijkeren, J. C. H. (2004). Modeling the in vitro intrinsic clearance of the slowly metabolized compound tolbutamide determined in sandwich-cultured rat hepatocytes. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 32*(8), 884–891. https://doi.org/10.1124/dmd.32.8.884

100. Tsai, J., Qiu, W., Kohen-Avramoglu, R., & Adeli, K. (2007). MEK-ERK inhibition corrects the defect in VLDL assembly in HepG2 cells: Potential role of ERK in VLDL-ApoB100 particle assembly. *Arteriosclerosis, Thrombosis, and Vascular Biology, 27*(1), 211–218. https://doi.org/10.1161/01.ATV.0000249861.80471.96

101. Van den Hof, W. F. P. M., Coonen, M. L. J., van Herwijnen, M., Brauers, K., Wodzig, W. K. W. H., van Delft, J. H. M., & Kleinjans, J. C. S. (2014). Classification of Hepatotoxicants Using HepG2 Cells: A Proof of Principle Study. *Chemical Research in Toxicology, 27*(3), 433–442. https://doi.org/10.1021/tx4004165

102. van Grunsven, L. A. (2017). 3D in vitro models of liver fibrosis. *Advanced Drug Delivery Reviews, 121*, 133–146. https://doi.org/10.1016/j.addr.2017.07.004

103. Waldherr, M., Mišík, M., Ferk, F., Tomc, J., Žegura, B., Filipič, M., Mikulits, W., Mai, S., Haas, O., Huber, W. W., Haslinger, E., & Knasmüller, S. (2018). Use of HuH6 and other human-derived hepatoma lines for the detection of genotoxins: A new hope for laboratory animals? *Archives of Toxicology, 92*(2), 921–934. https://doi.org/10.1007/s00204-017-2109-4

104. Wang, H., Xu, T., & Yin, D. (2023). Emerging trends in the methodology of environmental toxicology: 3D cell culture and its applications. *Science of The Total Environment, 857*, 159501. https://doi.org/10.1016/j.scitotenv.2022.159501

105. Wang, J.-Z., Xiong, N.-Y., Zhao, L.-Z., Hu, J.-T., Kong, D.-C., & Yuan, J.-Y. (2018). Review fantastic medical implications of 3D-printing in liver surgeries, liver regeneration, liver transplantation and drug hepatotoxicity testing: A review. *International Journal of Surgery, 56*, 1–6. https://doi.org/10.1016/j.ijsu.2018.06.004

106. Wu, X., Jiang, D., Yang, Y., Li, S., & Ding, Q. (2023). Modeling drug-induced liver injury and screening for anti-hepatofibrotic compounds using human PSC-derived organoids. *Cell Regeneration, 12*(1), 6. https://doi.org/10.1186/s13619-022-00148-1

107. Xie, F., Sun, L., Pang, Y., Xu, G., Jin, B., Xu, H., Lu, X., Xu, Y., Du, S., Wang, Y., Feng, S., Sang, X., Zhong, S., Wang, X., Sun, W., Zhao, H., Zhang, H., Yang, H., Huang, P., & Mao, Y. (2021). Three-dimensional bio-printing of primary human hepatocellular carcinoma for personalized medicine. *Biomaterials, 265*, 120416. https://doi.org/10.1016/j.biomaterials.2020.120416

108. Yang, S., Chen, Z., Cheng, Y., Liu, T., Lihong Yin, Pu, Y., & Liang, G. (2021). Environmental toxicology wars: Organ-on-a-chip for assessing the toxicity of environmental pollutants. *Environmental Pollution, 268*, 115861. https://doi.org/10.1016/j.envpol.2020.115861

109. Yang, Y., Yu, Z., Lu, X., Dai, J., Zhou, C., Yan, J., Wang, L., Wang, Z., & Zang, J. (2023). Minimally invasive bioprinting for in situ liver regeneration. *Bioactive Materials, 26*, 465–477. https://doi.org/10.1016/j.bioactmat.2023.03.011

110. Ye, S., Boeter, J. W. B., Penning, L. C., Spee, B., & Schneeberger, K. (2019). Hydrogels for Liver Tissue Engineering. *Bioengineering, 6*(3), 59. https://doi.org/10.3390/bioengineering6030059

111. Youdim, K. A., Tyman, C. A., Jones, B. C., & Hyland, R. (2007). Induction of Cytochrome P450: Assessment in an Immortalized Human Hepatocyte Cell Line (Fa2N4) Using a Novel Higher Throughput Cocktail Assay. *Drug Metabolism and Disposition, 35*(2), 275–282. https://doi.org/10.1124/dmd.106.012864

112. Zeigerer, A., Wuttke, A., Marsico, G., Seifert, S., Kalaidzidis, Y., & Zerial, M. (2017). Functional properties of hepatocytes in vitro are correlated with cell polarity maintenance. *Experimental Cell Research, 350*(1), 242–252. https://doi.org/10.1016/j.yexcr.2016.11.027

113. Zeilinger, K., Freyer, N., Damm, G., Seehofer, D., & Knöspel, F. (2016). Cell sources for in vitro human liver cell culture models. *Experimental Biology and Medicine, 241*(15), 1684–1698. https://doi.org/10.1177/1535370216657448

114. Zheng, P., Xu, Y., Ren, Z., Wang, Z., Wang, S., Xiong, J., Zhang, H., & Jiang, H. (2021). Toxic Prediction of Pyrrolizidine Alkaloids and Structure-Dependent Induction of Apoptosis in HepaRG Cells. *Oxidative Medicine and Cellular Longevity, 2021*, 1–12. https://doi.org/10.1155/2021/8822304

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