Cytocine™

Primary Porcine Hepatocytes

A Reliable and Cost-Effective Solution for Preclinical Research

CYTOTHERYX

Cytocine™ primary porcine hepatocytes offer a valuable alternative to human hepatocytes for a wide range of preclinical research applications, including drug development, toxicology, and liver disease studies. Pigs offer advantages to other non-human models, such as rodents, non-human primates, and dogs, due to their physiological and metabolic similarity to humans. Additionally, regulatory and ethical concerns support their use as a complementary model to human hepatocytes.

With high viability, plateability, metabolic functionality, and scalability, Cytocine™ hepatocytes provide a cost-effective and reliable solution for researchers who need a consistent supply of cells for their experiments. As we expand our offerings to include primary human hepatocytes, Cytotheryx is committed to providing the biomedical research community with the highest quality liver cells to support the development of new therapies, advance toxicological research, and improve our understanding of liver disease.

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The Role of Hepatocytes in Biomedical Research

Hepatocytes, the functional cells of the liver, are responsible for performing a wide array of essential physiological roles, including metabolism, detoxification, and biosynthesis of vital proteins (*e.g.*, albumin, transferrin, and numerous blood clotting factors)[1]. These cells, constituting roughly 80% of the liver's mass, are the engines that drive nutrient processing, breakdown of harmful substances, and bile production to aid digestion. Their ability to regulate lipid and carbohydrate metabolism, detoxify endogenous and exogenous compounds, and maintain organismal homeostasis makes hepatocytes indispensable for understanding and replicating liver function in biomedical research[2].

Hepatocytes play a pivotal role in preclinical drug studies and liver disease research. The liver is a primary site of both drug efficacy and toxicity; therefore, hepatocytes are used to evaluate how drugs are metabolized through the activity of cytochrome P450 (CYP450) enzymes[3,4], predict drug-drug interactions, assess drug clearance rates, and identify toxic metabolites. As an *in vitro* model system, they can reduce the risk of liver toxicity *in vivo* as candidate compounds progress to clinical trials[5]. Additionally, hepatocytes are critical in the study of liver diseases, ranging from viral hepatitis[6,7] to metabolic dysfunction-associated steatotic liver disease (MASLD)[8] and multiple end-stage liver diseases[9]. In these contexts, hepatocytes are used to model disease progression, identify potential therapeutic targets, and test the efficacy of novel treatments[10]. Hepatocytes are also being investigated for cell-based therapies as an alternative to costly and invasive orthotopic liver transplants[11-16].

Primary human hepatocytes are considered the gold standard for such studies due to their ability to precisely replicate human liver physiology, unlike transformed cell lines (*e.g.*, HepG2) or iPSC-derived hepatocyte-like cells[17-19]. However, their use is often constrained by limited availability, donor-to-donor variability, and poor post-thaw viability following cryopreservation. Commercially available primary human hepatocytes are typically procured from donated livers that are unsuitable for transplantation, leading to inconsistent supply and variable quality between lots. Moreover, variability in source material and cryopreservation protocols can compromise post-thaw functionality, such as cell viability and metabolic capacity, limiting their use for research and therapeutic applications.

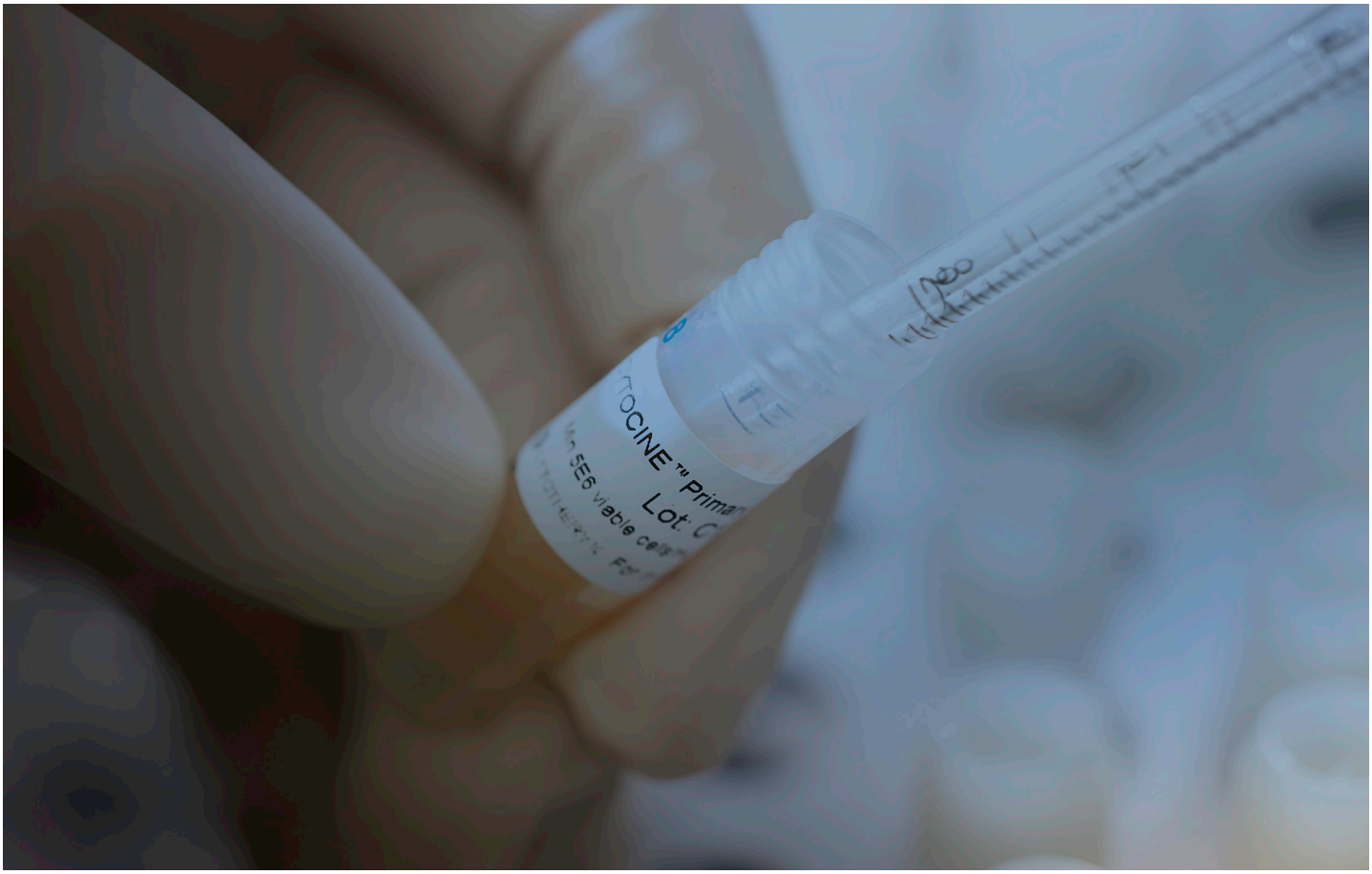
In response to the challenges with sourcing and reliability, as well as regulatory requirements for preclinical testing of new drugs in animal systems, researchers have long relied upon non-human models, including rodent (mice and rats) and non-rodent systems (*e.g.*, non-human primates (NHPs), rabbits, and dogs)[20]. These options each come with their own limitations. Rodents, while useful in some respects, do not adequately mimic human physiology, limiting their relevance in certain preclinical studies[21]. Rats exhibit the most divergent profile of CYP450 activity for commonly used animal species when compared to humans[22]. Additionally, the large-scale isolation of primary hepatocytes from dogs and NHPs is costly and fraught with ethical concerns, making these models less desirable for research purposes[23-25].

Cytocine™ Cryopreserved Primary Porcine Hepatocytes

Cytotheryx is pleased to offer Cytocine™ cryopreserved primary porcine hepatocytes, which provide researchers with a reliable, scalable, and cost-effective non-human model of primary hepatocytes that can be used for various biomedical research applications.

Our cryopreserved porcine hepatocytes are produced from an exceptionally high-health herd of animals in a highly controlled process to ensure superior cell viability and functionality upon thawing. Each lot is carefully processed to maintain critical metabolic activity, making them suitable for a wide range of *in vitro* assays and preclinical research. Additionally, our proprietary cryopreservation methods ensure that the cells are viable, plateable, and retain their full range of functions, including drug metabolism, detoxification, and protein synthesis, even after long-term storage.

Cytocine™ porcine hepatocytes retain their full range of metabolic functions post-thaw, including cytochrome P450 enzyme inducibility and activity.



Key Features of Cytocine™ Cryopreserved Porcine Hepatocytes

High Viability and Functionality

Cytocine™ cryopreserved porcine hepatocytes exhibit high viability (>75%) and plateability upon thawing, and retain their metabolic and functional capabilities for extended periods post-thaw. This makes our cells ideal for long-term studies and ensures reproducibility across experiments. We utilize advanced cryopreservation techniques to minimize cell damage during freezing and thawing, producing cells that perform reliably in various applications (Fig. 2). Cells are additionally tested for their ability to form spheroids, which are critical to 3D applications.

Batch Consistency

Consistency is crucial in scientific research, and Cytotheryx ensures that each lot of Cytocine™ hepatocytes undergoes rigorous quality control testing. This guarantees that researchers receive a consistent supply of cells with predictable characteristics, reducing variability and enabling reproducible results across multiple studies.

Scalability

Cytocine™ porcine hepatocytes can be produced at scale. Unlike animal hepatocytes from other suppliers, we offer a practical and reliable option for researchers requiring large quantities of hepatocytes for high-throughput screening, toxicology testing, or therapeutic applications. Cytotheryx can produce and supply large volumes of porcine hepatocytes, ensuring that researchers have continuous access to the cells they need.

Cryopreserved for Convenience

Our porcine hepatocytes are cryopreserved to give researchers the flexibility to store and use the cells as needed. Cryopreservation ensures that cells are available on demand, eliminating the need to source fresh tissue and reducing the logistical challenges associated with live cell procurement. This also makes our cells more cost-effective by reducing waste and ensuring that only the necessary quantity of cells is thawed for each experiment.

Full Metabolic Functionality

Cytocine™ porcine hepatocytes retain their full range of metabolic functions post-thaw, including cytochrome P450 enzyme inducibility (Fig. 1) and activity, protein biosynthetic function (*e.g.*, albumin production), and ammonia detoxification. This makes them highly relevant for drug metabolism studies, toxicology testing, and the development of liver disease models. Our cells are particularly well-suited for evaluating potential hepatotoxicity and assessing the metabolic fate of pharmaceutical compounds.

Technical Expertise and Support

The scientific staff at Cytotheryx is available to provide guidance to ensure optimal usage and applications for research purposes. Cytotheryx also offers thawing, plating and maintenance media customized for porcine hepatocytes to ensure optimal performance.

Advantages of Porcine Hepatocytes as a Non-Human Model

Pigs are recognized as a valuable model for human physiology due to their physiological and metabolic similarity to humans[26]. Porcine hepatocytes are readily available, scalable, and share important metabolic characteristics with human hepatocytes, particularly in terms of liver metabolism and enzyme expression[27].

Porcine hepatocytes are highly valued for their physiological similarity to human hepatocytes.

Furthermore, as the field of xenotransplantation develops and the number of associated preclinical studies using pigs increases, porcine hepatocytes provide a practical solution for bridging basic and translational research.

Porcine hepatocytes present several advantages over hepatocytes from other non-human species, particularly in terms of scalability, availability, and metabolic similarity, making them an increasingly valuable resource in biomedical research. As pigs are more widely recognized as a reliable model for human physiology, the use of porcine hepatocytes can advance various research fields.

One of the key advantages of porcine hepatocytes is their scalability. As a large animal, pigs can provide a significant quantity of hepatocytes from a single liver. The scale at which porcine hepatocytes are offered is essential for research projects that require large numbers of cells, such as high-throughput screening, toxicology testing, or therapeutic applications.

Unlike human hepatocytes, which are limited by the scarcity of donor organs, porcine hepatocytes can be produced in large lot sizes with unparalleled consistency, ensuring a reliable and steady supply for ongoing research initiatives.

Additionally, porcine hepatocytes are highly valued for their physiological similarity to human hepatocytes. Pigs share many of the same metabolic pathways and liver enzyme activities as humans, particularly the spectrum of cytochrome P450 enzymes responsible for drug metabolism (Fig 1.).

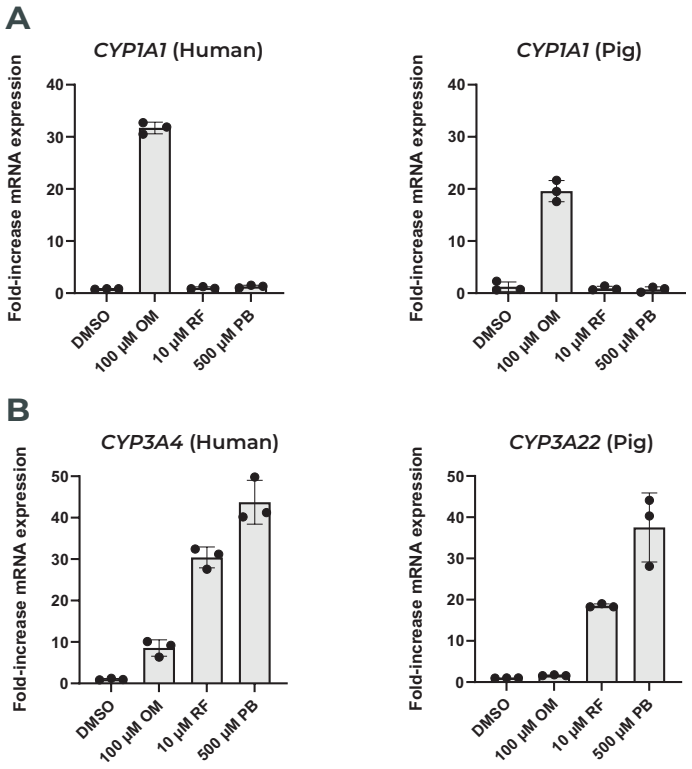


Figure 1. Cytocine™ **Hepatocytes Model Human CYP450 Induction**. (A) Comparison of mRNA fold-induction of *CYP1A1* isoforms in primary human hepatocytes (left) and Cytocine™ lot 1179 primary porcine hepatocytes (right). Cryopreserved cells were cultured on collagen-coated plates for 24 h with omeprazole (100 µM), rifampicin (10 µM), or phenobarbital (500 µM). Experiments were performed in triplicate. Error bars represent mean +/- SD. (B) Comparison of fold-induction of *CYP3A4* isoforms in primary human hepatocytes (left) and Cytocine™ lot 1179 primary porcine hepatocytes (right). Experiments were performed in triplicate. Error bars represent mean +/- SD
Note: Porcine *CYP3A22* is orthologous to human *CYP3A4*

Advantages of Porcine Hepatocytes as a Non-Human Model

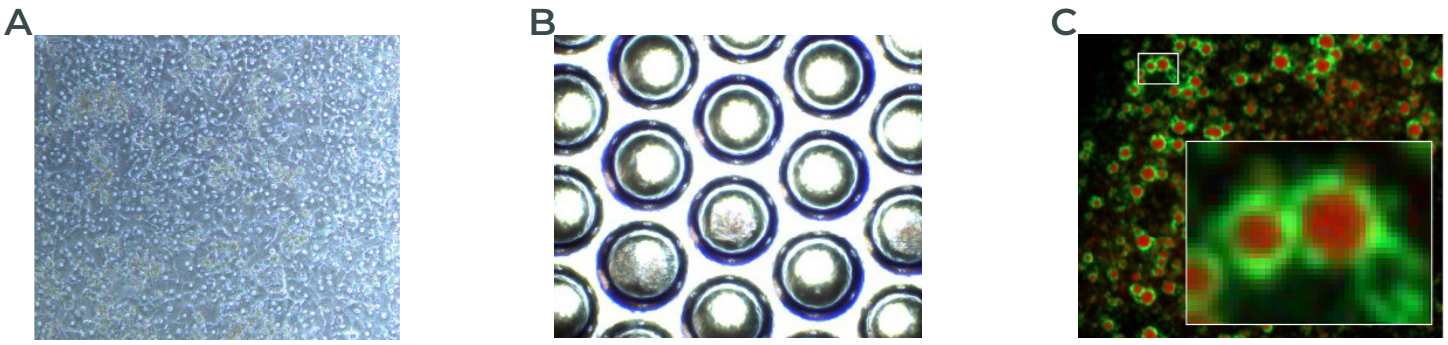


Figure 2. Applications for Cytocine™ Hepatocytes. (A) Cytocine™ lot C1193, 48 h post-thaw (w/ 2% Geltrex overlay) shows confluent plating with characteristic cobblestone morphology. (B) Cytocine™ lot C1179, 24 h post-thaw cultured in Corning® Elplasia® 6-well round-bottom microcavity plate demonstrates capability of 3D spheroid formation. (C) Immunofluorescence microscopy on fixed Cytocine™ lot C1149 hepatocytes 24 h post-thaw showing staining of a key regulator of hepatic lipid metabolism (HSD17β13, green) surrounding intracellular triglyceride-enriched lipid droplets (Oil Red O staining).

Many of the same subfamilies of the human P450 system are intact in pigs, including the *CYP1A, CYP2A, CYP2C, CYP2D, CYP2E*, and *CYP3A* subfamilies[25,28]. Moreover, the sequence homology between pig and human P450 isoforms ranges from 72% to 95%, with a similarity of approximately 98%[28,29]. This positions pigs as a superior model compared to rodents, whose metabolic pathways often diverge significantly from those of humans.

Pigs also surpass dogs as a model for liver studies due to additional similarities to humans. Unlike dogs, which lack key enzymes like aldehyde oxidase (AOX) and N-acetyltransferases (NAT1 and NAT2), pigs possess these enzymes, making them more comparable to humans in metabolic studies[30,31]. Studies on pigs have shown that their *in vivo* clearance of AOX substrates closely parallels that of humans, supporting their use in pharmacokinetic modeling[25.]

Furthermore, pigs exhibit a high degree of similarity in phase II metabolic processes, such as glucuronidation, when compared to humans. Studies have shown that pigs possess enzymes like UDP-glucuronosyltransferase and glucuronidate drugs in ways comparable to the human liver[25,32].

This makes porcine hepatocytes an excellent model for studying drug metabolism, liver function, and toxicology, closely mimicking the processes that occur in the human liver.

Cost-effectiveness is another significant benefit of porcine hepatocytes. Compared to the limited availability and high cost of human hepatocytes, porcine hepatocytes are much more affordable. Their ease of production and consistent availability make them a cost-effective option for both academic institutions and industry. For researchers working with limited budgets, porcine hepatocytes provide an accessible, scalable alternative that retains functional integrity.

Finally, porcine hepatocytes align with the growing regulatory support for xenotransplantation. The FDA has increasingly recognized xenotransplantation as a promising solution to the organ donor shortage. Because pigs are the most prevalent animal model in xenotransplantation research, porcine hepatocytes offer a robust platform for developing a better understanding of porcine metabolic processes.

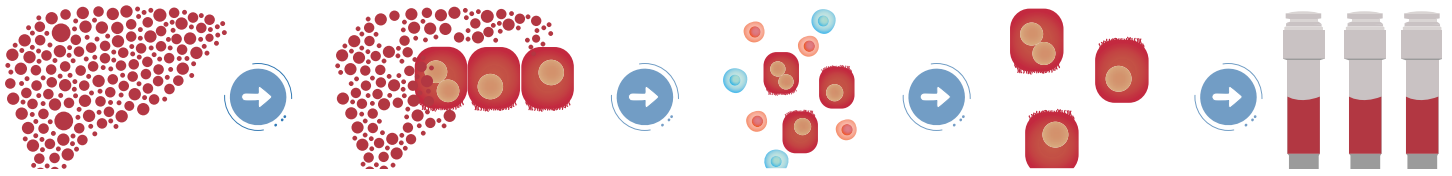
Future Developments: Cytoprime™ Primary Human Hepatocytes

While porcine hepatocytes provide a valuable model for many research applications, we recognize that human hepatocytes remain the gold standard for drug metabolism, toxicology, and liver disease studies. Cytotheryx is developing a scalable source of primary human hepatocytes, which will soon be available to complement our porcine hepatocyte offerings.

Our proprietary bio-incubator technology will enable us to provide researchers with a consistent and reliable supply of human liver cells. This innovative approach addresses the limitations of donor variability and scarcity, offering a scalable solution for producing primary human hepatocytes that retain full metabolic function.

Our process begins with careful perfusion and enzymatic dissociation of donor liver tissue. Proprietary techniques allow for the subsequent enrichment of a highly purified population of primary hepatocytes. These cells are then transplanted into our bio-incubator, an *in vivo* system with the capacity to expand cells greater than 1000-fold. Expanded human hepatocytes are selectively isolated and supplied fresh or cryopreserved for future use. This cutting-edge process ensures that researchers in both academic and industrial settings have access to the highest quality plateable and ready-to-use primary human hepatocytes on the market.

By offering both porcine and human hepatocytes, Cytotheryx will give researchers the flexibility to choose the most appropriate model for their specific needs. Whether studying drug metabolism, liver disease, or developing novel therapies, our cells will offer a reliable and scalable solution for advancing biomedical research.



References

1 Schulze, R. J., Schott, M. B., Casey, C. A., Tuma, P. L. & McNiven, M. A. The cell biology of the hepatocyte: A membrane trafficking machine. J Cell Biol 218, 2096-2112 (2019). https://doi.org/10.1083/jcb.201903090

2 Vernetti, L. A., Vogt, A., Gough, A. & Taylor, D. L. Evolution of Experimental Models of the Liver to Predict Human Drug Hepatotoxicity and Efficacy. Clin Liver Dis 21, 197-214 (2017). https://doi.org/10.1016/j.cld.2016.08.013

3 Esteves, F., Rueff, J. & Kranendonk, M. The Central Role of Cytochrome P450 in Xenobiotic Metabolism-A Brief Review on a Fascinating Enzyme Family. J Xenobiot 11, 94-114 (2021). https://doi.org/10.3390/jox11030007

4 Guengerich, F. P. A history of the roles of cytochrome P450 enzymes in the toxicity of drugs. Toxicol Res 37, 1-23 (2021). https://doi.org/10.1007/s43188-020-00056-z

5 Hewitt, N. J. et al. Primary hepatocytes: current understanding of the regulation of metabolic enzymes and transporter proteins, and pharmaceutical practice for the use of hepatocytes in metabolism, enzyme induction, transporter, clearance, and hepatotoxicity studies. Drug Metab Rev 39, 159-234 (2007). https://doi.org/10.1080/03602530601093489

6 Allweiss, L. & Dandri, M. Experimental *in vitro* and *in vivo* models for the study of human hepatitis B virus infection. J Hepatol 64, S17-S31 (2016). https://doi.org/10.1016/j.jhep.2016.02.012

7 Zhang, Z. et al. Hepatitis B Virus Particles Activate Toll-Like Receptor 2 Signaling Initially Upon Infection of Primary Human Hepatocytes. Hepatology 72, 829-844 (2020). https://doi.org/10.1002/hep.31112

8 Jun, J. H. et al. The senescence-associated secretome of hedgehog-deficient hepatocytes drives MASLD progression. J Clin Invest (2024). https://doi.org/10.1172/JCI180310

9 Bhogal, R. H. et al. Isolation of primary human hepatocytes from normal and diseased liver tissue: a one hundred liver experience. PLoS One 6, e18222 (2011). https://doi.org/10.1371/journal.pone.0018222

10 Vinken, M. Primary hepatocyte cultures for liver disease modeling. Current Opinion in Toxicology 25, 1-5 (2021). https://doi.org/10.1016/j.cotox.2020.08.003

11 Sun, Z. et al. Hepatocyte transplantation: The progress and the challenges. Hepatol Commun 7 (2023). https://doi.org/10.1097/HC9.0000000000000266

12 Hu, X. H. et al. Cell therapy in end-stage liver disease: replace and remodel. Stem Cell Res Ther 14, 141 (2023). https://doi.org/10.1186/s13287-023-03370-z

13 Dwyer, B. J., Macmillan, M. T., Brennan, P. N. & Forbes, S. J. Cell therapy for advanced liver diseases: Repair or rebuild. J Hepatol 74, 185-199 (2021). https://doi.org/10.1016/j.jhep.2020.09.014

14 Iansante, V., Mitry, R. R., Filippi, C., Fitzpatrick, E. & Dhawan, A. Human hepatocyte transplantation for liver disease: current status and future perspectives. Pediatr Res 83, 232-240 (2018). https://doi.org/10.1038/pr.2017.284

15 Huebert, R. C. & Rakela, J. Cellular therapy for liver disease. Mayo Clin Proc 89, 414-424 (2014). https://doi.org/10.1016/j.mayocp.2013.10.023

16 Dhawan, A., Puppi, J., Hughes, R. D. & Mitry, R. R. Human hepatocyte transplantation: current experience and future challenges. Nat Rev Gastroenterol Hepatol 7, 288-298 (2010). https://doi.org/10.1038/nrgastro.2010.44

17 Blaszkiewicz, J. & Duncan, S. A. Advancements in Disease Modeling and Drug Discovery Using iPSC-Derived Hepatocyte-like Cells. Genes (Basel) 13 (2022). https://doi.org/10.3390/genes13040573

18 Lu, J. et al. Morphological and Functional Characterization and Assessment of iPSC-Derived Hepatocytes for *In Vitro* Toxicity Testing. Toxicol Sci 147, 39-54 (2015). https://doi.org/10.1093/toxsci/kfv117

19 Bonanini, F. et al. A comparison between different human hepatocyte models reveals profound differences in net glucose production, lipid composition and metabolism *in vitro*. Exp Cell Res 437, 114008 (2024). https://doi.org/10.1016/j.yexcr.2024.114008

20 Dirven, H. et al. Performance of preclinical models in predicting drug-induced liver injury in humans: a systematic review. Sci Rep 11, 6403 (2021). https://doi.org/10.1038/s41598-021-85708-2

21 Hammer, H., Schmidt, F., Marx-Stoelting, P., Potz, O. & Braeuning, A. Cross-species analysis of hepatic cytochrome P450 and transport protein expression. Arch Toxicol 95, 117-133 (2021). https://doi.org/10.1007/s00204-020-02939-4

22 Turpeinen, M. et al. Predictive value of animal models for human cytochrome P450 (CYP)-mediated metabolism: a comparative study *in vitro*. Xenobiotica 37, 1367-1377 (2007). https://doi.org/10.1080/00498250701658312

23 Son, Y. W., Choi, H. N., Che, J. H., Kang, B. C. & Yun, J. W. Advances in selecting appropriate non-rodent species for regulatory toxicology research: Policy, ethical, and experimental considerations. Regul Toxicol Pharmacol 116, 104757 (2020). https://doi.org/10.1016/j.yrtph.2020.104757

24 Walker, R. L. & Eggel, M. From Mice to Monkeys? Beyond Orthodox Approaches to the Ethics of Animal Model Choice. Animals (Basel) 10 (2020). https://doi.org/10.3390/ani10010077

25 Tang, H. & Mayersohn, M. Porcine Prediction of Pharmacokinetic Parameters in People: A Pig in a Poke? Drug Metab Dispos 46, 1712-1724 (2018). https://doi.org/10.1124/dmd.118.083311

26 Bode, G. et al. The utility of the minipig as an animal model in regulatory toxicology. J Pharmacol Toxicol Methods 62, 196-220 (2010). https://doi.org/10.1016/j.vascn.2010.05.009

27 Gerbal-Chaloin, S., Briolotti, P., Daujat-Chavanieu, M. & Rasmussen, M. K. Primary hepatocytes isolated from human and porcine donors display similar patterns of cytochrome p450 expression following exposure to prototypical activators of AhR, CAR and PXR. Curr Res Toxicol 2, 149-158 (2021). https://doi.org/10.1016/j.crtox.2021.03.002

28 Achour, B., Barber, J. & Rostami-Hodjegan, A. Cytochrome P450 Pig liver pie: determination of individual cytochrome P450 isoform contents in microsomes from two pig livers using liquid chromatography in conjunction with mass spectrometry [corrected]. Drug Metab Dispos 39, 2130-2134 (2011). https://doi.org/10.1124/dmd.111.040618

29 Anzenbacher, P. et al. Presence and activity of cytochrome P450 isoforms in minipig liver microsomes. Comparison with human liver samples. Drug Metab Dispos 26, 56-59 (1998).

30 Garattini, E. & Terao, M. Aldehyde oxidase and its importance in novel drug discovery: present and future challenges. Expert Opin Drug Discov 8, 641-654 (2013). https://doi.org/10.1517/17460441.2013.788497

31 Diaz, G. J. & Squires, E. J. Role of aldehyde oxidase in the hepatic *in vitro* metabolism of 3-methylindole in pigs. J Agric Food Chem 48, 833-837 (2000). https://doi.org/10.1021/jf990805u

32 Dalgaard, L. Comparison of minipig, dog, monkey and human drug metabolism and disposition. J Pharmacol Toxicol Methods 74, 80-92 (2015). https://doi.org/10.1016/j.vascn.2014.12.005

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