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Human Liver Organoids for Assessing Anticancer Drug Hepatotoxicity

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Abstract. Human liver organoids (HLOs) are increasingly being developed as human-relevant platforms for preclinical hepatotoxicity assessment, but their suitability for anticancer drug testing remains incompletely established. This review examines how far current HLO systems can support prediction, mechanistic analysis and testing under more complex treatment conditions. Evidence reviewed shows that HLOs can reproduce clinically relevant drug induced liver injury (DILI) responses, distinguish compounds with different hepatotoxic profiles and provide functional readouts beyond simple cytotoxicity. Their application has also extended to drug-drug interactions and immune-mediated injury, demonstrating potential in scenarios that challenge conventional in vitro models. However, predictive performance varies between HLO systems, while reproducibility, standardization and incomplete physiological and immune complexity continue to limit broader translation. Overall, HLOs represent a promising but still developing platform for anticancer hepatotoxicity assessment. Future work should broaden functional competence, improve cross-platform reliability and progressively integrate immune or even multi-organ interactions to support more comprehensive prediction of human treatment-related toxicity responses.

Keywords: Human liver organoids, Drug-induced liver injury, Anticancer drugs, Drug-drug interactions, Immune-mediated liver injury

1. Introduction

Development of anticancer drugs has significantly advanced cancer treatment, but treatment-related toxicity remains an inescapable limitation. The liver, which plays a central role in drug metabolism and elimination, is often highly exposed to parent drugs and their metabolites, making it susceptible to DILI. In order to minimize risks to patients and avoid toxicity-related dose reduction, treatment interruption or discontinuation, preclinical toxicity assessment is crucial before anticancer drug candidates enter clinical trials. Therefore, reliable prediction of anticancer drug-induced hepatotoxicity is essential for both patient safety and effective cancer treatment.

When predicting such hepatotoxicity, animal models, 2D hepatic cell lines and primary human hepatocytes (PHHs) are commonly used, yet none of these systems provides an ideal solution. Although the animal models can simulate whole-body physiology, they are still limited by species-

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specific differences in metabolism and susceptibility. 2D hepatic cell lines are convenient to culture, making a good choice for large scale screening, but the microenvironment, polarity and cell-cell interactions can be oversimplified with part of the metabolic activity reduced. PHHs provide greater human relevance, however the limited donor supply, poor proliferation capacity and the rapid loss of differentiated functions during in vitro culture remain unsolved. Collectively, these trade-offs force researchers to choose between systemic complexity, experimental convenience and direct human relevance [1].

In response, human liver organoids (HLOs) have emerged as human-relevant 3D models capable of reproducing selected hepatic functions involved in xenobiotic metabolism and toxicity [2, 3]. Recent studies have also shown that liver organoids can be applied to anticancer drug-associated hepatotoxicity under more complex treatment contexts involving combined drug exposure and potential drug-drug interactions [4]. Beyond such pharmacological interactions, cancer treatment-associated hepatotoxicity may also involve treatment-induced immune responses rather than direct drug-hepatocyte interactions alone [5]. The ability of HLO-based systems to incorporate immune components and reproduce such responses highlights their capacity to model more complex forms of treatment-related hepatotoxicity [6].

Although liver organoids have been increasingly investigated for general DILI prediction [2, 3], evidence specifically concerning anticancer-drug hepatotoxicity remains limited, while differences in model standardization and the separation of functional capabilities across HLO platforms continue to restrict broader application. This review therefore evaluates the current evidence for HLO-based anticancer hepatotoxicity assessment, with particular attention to predictive performance, mechanistic relevance, complex treatment contexts and the remaining barriers to broader preclinical application.

2. Principles and application of HLOs

2.1. Principles and characteristics of HLOs

HLOs are three-dimensional, self-organizing human cell culture systems derived from liver tissue or stem-cell sources with the potential to reproduce selected structural and functional features of the human liver. In 2019, Mun et al. generated hepatocyte-like liver organoids from human pluripotent stem-cells (PSCs), which were self-renewing, highly proliferative, and maturation-competent, while also being suitable for long-term storage with a high viability remaining after cryopreservation [7]. The liver-like characteristics of these PSC-derived HLOs were reflected in both their structural organization and hepatic functions. Yet, CYP3A4 and CYP1A2 activities remained below those observed in PHHs, indicating that hepatocyte maturation remains incomplete in this model [7]. At the structural level, Shinozawa et al. demonstrated that PSC-derived HLOs formed polarized bile canaliculi-like structures capable of unidirectional bile acid transport [2]. Functionally, Mun et al.'s organoids retained multiple mature hepatic functions, including glycogen storage, indocyanine green uptake and release, as well as inducible CYP3A4 activity and functional drug metabolism [7]. Although these organoid systems did not fully reach the maturity of PHHs, particularly in their drug-metabolizing enzyme activity, their cellular polarity, bile canaliculi-like structures and retained hepatic functions still provide a biological basis for HLO-based hepatotoxicity testing.

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2.2. Applications and readouts in hepatotoxicity assessment

For HLOs to become practical drug-screening platforms, they need to balance experimental scalability with the retention of liver-specific functions. To address these competing requirements, two complementary HLO application formats were developed by Zhang et al., in which the 384-well platform was adapted for rapid high-throughput screening, whereas the patient-derived liver-on-chip (PaDLOC) system was used for more physiologically relevant characterization of liver-specific functions and toxicity responses [3]. For the 384-well format, its screening role was supported by the reproducible dose-dependent cytotoxicity assessment together with the morphological profiling that distinguished drug-specific phenotypic responses [3]. On the other hand, the PaDLOC system showed enhanced liver-specific functionality through increased albumin production and CYP450 expression, supporting its use for more physiologically relevant characterization [3]. Importantly, this enhanced liver-specific functionality was also reflected in toxicity-related readouts, as hepatotoxic drug exposure induced release of the liver injury biomarkers ALT and AST, together with reduced albumin production in the PaDLOC system [3]. Shin et al. further extended this multiparametric approach by combining multiple toxicity-related endpoints, including viability, mitochondrial, inflammatory and oxidative-stress responses, into a radar profile [8], illustrating a more comprehensive picture of how HLO-based assessment can be integrated into a single analysis with complementary dimensions of liver injury.

3. Evaluating liver organoids for anticancer drug-induced hepatotoxicity

3.1. Predictive relevance

While the previous section describes how HLOs can be applied and what toxicity-related readouts they provide, their value as a preclinical toxicity prediction model ultimately depends on how reliably these responses correspond to clinically observed DILI. This predictive relevance was evaluated on a larger scale by Shinozawa et al. [2], who developed a liver organoid-based toxicity screening system and tested it using 238 marketed drugs. Each drug was tested at four different concentrations and in order to do the predictive analysis, the HLO response at the tested concentration closest to but above its reported clinical Cmax was selected for further toxicity assessment. Based on the comparison between the HLO-based toxicity prediction and the compound's known clinical DILI classification, the model achieved 88.7% sensitivity and 88.9% specificity [2]. This indicates that most known DILI-positive compounds were correctly detected, and similarly high specificity shows that relatively few negative compounds were wrongly classified as hepatotoxic. However, different HLO systems can show different predictive performance. Bouwmeester et al. evaluated a different type of liver organoid, hepatocyte-like intrahepatic cholangiocyte organoids (HL-ICOs), and directly compared their responses with PHHs and the HepaRG hepatic cell line [9]. For acetaminophen (APAP), the EC50 in HL-ICOs was approximately 24.0-26.8mM, compared with 4.19mM in PHHs and 3.46-6.04mM in HepaRG cells [9]. This obviously lower responses of HL-ICOs to APAP challenges the assumption that organoid-based models necessarily provide greater predictive sensitivity than conventional hepatic models. A possible explanation for that may be the incomplete CYP-mediated APAP bioactivation in HL-ICOs as is this actually introducing the formation of the toxic APAP metabolite NAPQI [9]. However, this difference was not consistently observed across all tested compounds, suggesting that the predictive advantage of HLOs still exists, but may depend on both the compound and the capability of the specific organoid model.

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3.2. Mechanistic relevance

The liver performs multiple functions involved in drug handling and cellular homeostasis including xenobiotic metabolism, bile transport and mitochondrial activity. As DILI can result in disruption of different hepatic processes, changes in these functions may provide clues to the mechanisms underlying the observable toxicity, and since HLOs retain several of these liver-like functions, their responses may reveal that. Therefore, building on Shinozawa et al.'s PSC-derived HLO models with polarized bile canaliculi-like architecture, both bile-transport inhibition and mitochondrial stress are assessed [2]. Notably, the results did not show a simple one compound to one response pattern, overlapping responses were also observed. Moreover, drugs showing dual stress progressively produced even greater viability loss, suggesting that DILI severity may arise from the convergence of multiple mechanisms rather than one isolated pathway. Zhang et al. extended this approach by combining morphological profiling with single-cell transcriptomics [3]. Results showed the competence of characterizing the affected biological processes through the distinct phenotypic and molecular signatures produced by using different hepatotoxic drugs. Together, these studies suggest that HLOs can capture multiple mechanistic dimensions of DILI through complementary functional, phenotypic and molecular readouts.

3.3. Cancer-treatment-specific complexity: from direct to immune-mediated hepatotoxicity

In cancer treatment, hepatotoxicity assessment can become more complex due to that multiple drugs are often used simultaneously. This raises problems of whether the hepatotoxicity of individual drugs changes when they are used together and whether the HLOs could reproduce such interactions. Tan et al. examined this by analyzing the FAERS database and a much stronger DILI signal for ceritinib combination therapy than for ceritinib monotherapy can be seen. Among those tested ceritinib combinations, ceritinib-ibuprofen showed the strongest synergistic hepatotoxicity. Mechanistically, this can be explained by the suppression of CYP3A4 activity, which could cause impairing ceritinib metabolic clearance and increasing its hepatotoxic potential [4]. These findings suggest that the use of HLOs is able to extend beyond single-drug toxicity testing by capturing combination-dependent increases in hepatotoxic responses and providing mechanistic insight into the drug-drug interactions underlying such changes. However, evidence remains limited to the small number of drug combinations examined, so whether this capability can be generalized across anticancer treatments remains questionable.

Beyond drug-drug interactions, cancer-treatment-associated hepatotoxicity may also involve immune-mediated liver injury. Conventional HLOs reproduce selected liver-like characteristics but lack the adaptive immune components present in the clinical setting [6]. This highlights the need for immune-competent HLO-based models and El Abdellaoui Soussi et al. addressed this gap by co-culturing iPSC-derived HLOs with autologous CD8+ T cells [6]. By using flucloxacillin as a proof of concept, only marginal direct toxicity was observed when HLOs were exposed to the drug alone. However, when appropriately primed CD8+ T cells were added to the system, hepatocyte injury became evident, indicating that the co-culture model is capable of detecting immune-mediated DILI in vitro, which was previously undetectable in HLOs alone [6]. (Fig. 1)

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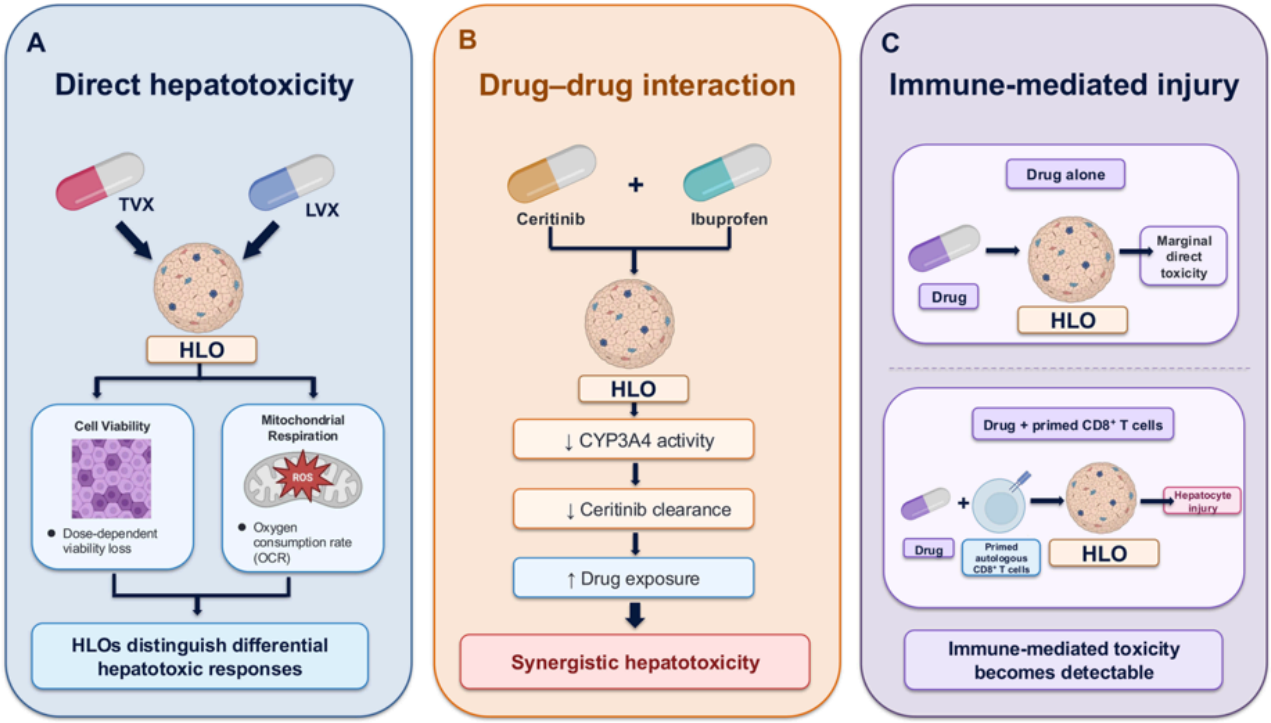

Figure 1. Increasing complexity of treatment-associated hepatotoxicity in HLO-based models. Panel A summarizes differential TVX/LVX toxicity [7]; panel B outlines ceritinib-ibuprofen synergistic hepatotoxicity [4]; panel C shows immune-mediated injury revealed by primed autologous CD8+ T-cell co-culture [6]. Created in BioRender. Dai, J. (2026) https://BioRender.com/zbvbcqc

4. Experimental evidence for liver organoids in hepatotoxicity prediction

4.1. Application in general drug-induced liver injury – trovafloxacin vs levofloxacin

Trovafloxacin (TVX), a fluoroquinolone antibiotic, has been withdrawn because of severe hepatotoxicity. However, levofloxacin (LVX), a structurally related analogue from the same fluoroquinolone family, is comparatively less toxic. In a particular study carried out by Mun et al., those two drugs were used to examine whether HLOs can not only detect drug-induced cytotoxicity but also distinguish between structurally related compounds with different levels of clinical hepatotoxicity [7]. As the TVX concentration increased, a significant reduction in HLO viability was observed at the TVX concentrations of 0.8 and 4 μM, both at and below its reported Cmax of 4.1 μM [7]. When the same test was performed with LVX, the concentration increase over the same exposure range did not cause cytotoxicity to the same extent as TVX, as LVX only reduced organoid viability at the highest tested concentration of 100 μM [7]. This differential response suggests that HLOs can distinguish clinically relevant hepatotoxicity between structurally related compounds rather than simply responding to nonspecific cytotoxicity. Meanwhile, the discriminatory response was also observed at the functional level. When XFe96 Flux Analyzer was used to measure the oxygen consumption rate (OCR), impaired mitochondrial respiration was observed at the same TVX concentrations of 0.8 and 4 μM, whereas LVX produced little comparable effect at the same concentrations [7]. This suggests that the HLO model could not only capture the loss of cell viability and distinguish between structurally related compounds but also provide insight into the possible underlying mechanism, such as mitochondrial dysfunction associated with TVX-induced

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hepatotoxicity. To further evaluate the performance of HLOs in preclinical toxicity testing, the response to TVX was also compared with that of conventional 2D mature hepatocytes. An evident reduction in cell number was only observed when the TVX concentration reached 20 μM, substantially exceeding its reported Cmax of 4.1 μM [7]. Hence, it can be concluded from this case that, for TVX, the 3D organoid model produced a toxicity response that was more consistent with its known clinical hepatotoxicity profile than the corresponding 2D system.

4.2. Application in anticancer combination therapy drug-induced liver injury – ceritinib

Building on the drug-drug interaction identified above, Tan et al. further examined whether HLOs could experimentally reproduce the enhanced hepatotoxicity associated with ceritinib combination therapy [4]. In this experiment, ibuprofen and APAP were selected for combination testing with ceritinib in HLOs. By applying the Bliss independence model to the combination data, Tan et al. found that both ceritinib-ibuprofen and ceritinib-APAP produced greater hepatotoxic effects than expected from independent drug action, indicating the presence of synergistic hepatotoxicity [4]. Because this synergistic toxicity cannot be reliably inferred from the effects of the individual drugs alone, directly testing the combination in HLOs becomes particularly important for identifying interaction-dependent hepatotoxicity. The ceritinib-ibuprofen combination produced the more pronounced synergistic response, consistent with its stronger pharmacovigilance signal (ROR 12.01) compared with ceritinib-APAP (ROR 5.82) [4]. This indicates that HLOs retained the ability to distinguish different magnitudes of hepatotoxicity even in the more complex setting of drug-drug interactions. Additionally, ibuprofen reduced CYP3A4 activity in the HLO model which may impair ceritinib metabolic clearance and thereby increase ceritinib exposure, providing a possible explanation for the stronger hepatotoxic response [4]. The ceritinib-ibuprofen case therefore provides an experimental example in which a clinically observed combination-dependent hepatotoxicity signal was reproduced in an HLO model.

5. Challenges and future perspectives

5.1. Technical barriers to translation

Although the potential of HLOs in hepatotoxicity testing has been demonstrated, several barriers still obstruct their routine use in large-scale drug screening. One such barrier is the heterogeneity of HLOs generated using conventional Matrigel dome culture, which Jiang et al. linked to the limited control over the organoid growth microenvironment [10]. Such variability may introduce experimental noise into viability-based toxicity measurements and reduce reproducibility between organoids. However, the usage of micro-patterned agarose scaffold allowed Jiang et al. to generate more than 8000 relatively uniform HLOs in a 48-well plate, demonstrating that greater control of organoid size and production is achievable. On top of that, scalability remains another limitation, as conventional Matrigel dome culture is relatively costly, labor-intensive and low throughput [11]. Shrestha et al. addressed this using pillar-plate microarray 3D bioprinting and as a result, the reproducible organoid generation with a coefficient of variation of approximately 15-18% was achieved while using substantially smaller culture volumes [11]. Functional standardization is also required as the term 'HLO' does not guarantee equivalent hepatic competence across different models. Choi et al. demonstrated a possible approach by validating key hepatic functions before toxicity testing and subsequently confirming that the qualified organoids could distinguish hepatotoxic from non-hepatotoxic compounds [12]. By doing this kind of pre-testing, functional

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qualification may therefore provide a practical route towards improving cross-platform standardization, although universally accepted qualification criteria still requiring establishment [12].

5.2. Future development towards immune-competent liver organoids

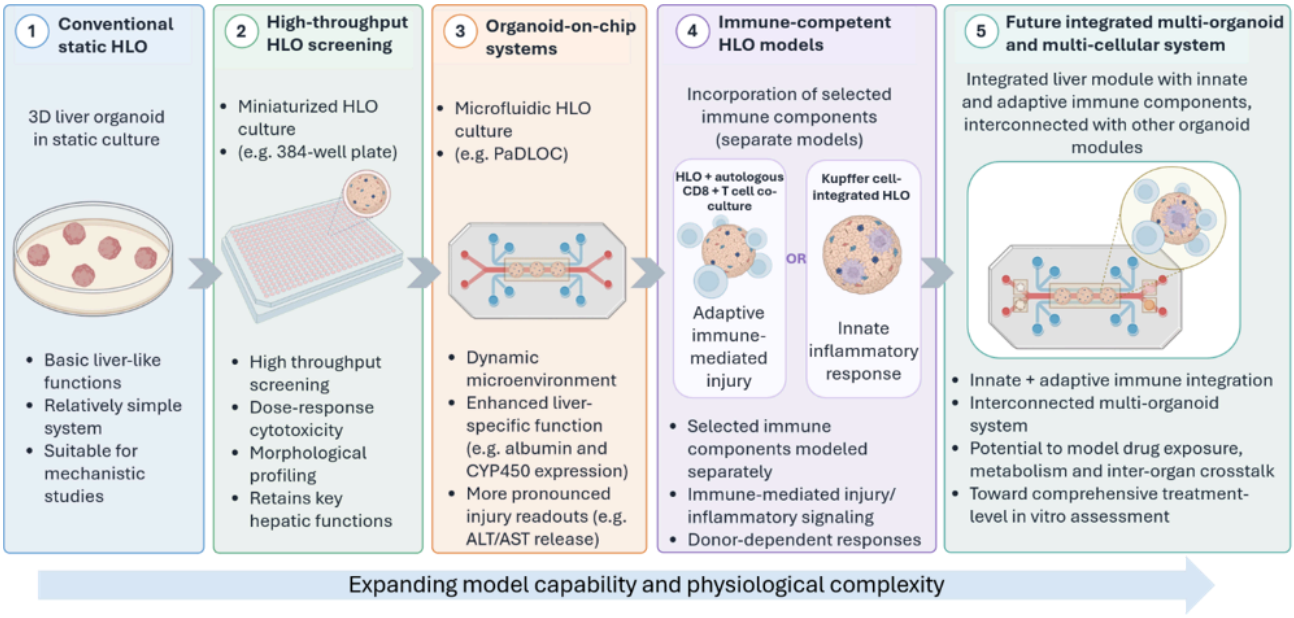

Figure 2. Evolution of HLO-based hepatotoxicity models towards increasing physiological complexity. Panels 1-3 present static, high-throughput and organoid-on-chip formats [3]; panel 4 shows separate CD8+ T-cell co-culture [6] and Kupffer cell-integrated HLO models [13]; panel 5 outlines a proposed multi-organoid system, with the enlarged inset showing the integrated liver module. Created in BioRender. Dai, J. (2026) https://BioRender.com/rinmhdq

Technical optimization alone, however, still cannot reproduce the dynamic multicellular environment of the human liver. The PaDLOC approach discussed earlier provides one route towards greater physiological complexity as Zhang et al.'s PaDLOC study demonstrated that HLO-based culture could move beyond the conventional static environment through microfluidic integration with continuous perfusion [3]. This increase in dynamic physiological complexity provides a useful basis for further addressing other biological limitations of HLO-based systems, including the finite representation of immune interactions, for example, the development of autologous CD8+ T cells co-culture discussed earlier. However, it only represents one component of the hepatic immune environment. Li et al. further extended this direction by incorporating Kupffer cells, a resident innate immune component, into liver organoids [13]. Unlike the CD8+ T-cell co-culture approach, Li et al. introduced erythro-myeloid progenitors during organoid formation, the Kupffer-like macrophages could therefore become integrated within the organoid environment. Following tests show that this measure could also trigger inflammatory responses, approving the feasibility of incorporating functional innate immune components into HLO-based models [13]. Together, these studies show that both adaptive and innate immune components are beginning to be represented in HLO-based systems, increasing their biological relevance for immune-mediated toxicity assessment. Nonetheless, they still only reproduce selected immune components rather than the coordinate immune environment present in vivo. If HLO-based models are planned to be applied

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extensively in anticancer treatment-associated hepatotoxicity testing, the reconstruct in immune complexity becomes particularly relevant, a single T-cell or macrophage population may still be insufficient to capture the multiple immune pathways involved in liver injury. Hence, future development may therefore require trying of the integration between innate and adaptive immune components, and if a more complete immune environment could be reproduced within an in vitro HLO system, its biological relevance for anticancer hepatotoxicity assessment would be further strengthened (Fig. 2).

6. Conclusion

Overall, current evidence shows that HLOs can reproduce clinically relevant hepatotoxic responses while providing mechanistic information beyond cytotoxicity alone. Their expansion from single-drug DILI assessment to drug-drug interactions and immune-mediated injury further highlights their relevance to anticancer treatment, where hepatotoxicity can involve complex interactions that remain difficult to reproduce in conventional in vitro models. However, technical standardization and reproducibility remain incomplete, while current HLOs reproduce only selected aspects of systemic and immune complexity showing that further development is still required at both the technical and biological levels. Hence, rather than serving as a universal replacement model at present, HLO platforms should be selected according to the testing context to make the best use of their individual strengths. In the longer term, integrating currently separated functional capabilities into more broadly competent HLOs, and eventually linking these models with other organ systems, may extend toxicity prediction from context-specific liver responses towards more comprehensive human-relevant assessment.

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