



INTEGRATIVE LIVER–KIDNEY ORGAN-ON-CHIP PLATFORM FOR MECHANISTIC EVALUATION OF DRUG-INDUCED NEPHROTOXICITY

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Abstract

Drug-induced nephrotoxicity is a paramount impediment in the drug development and clinical practice because it is closely linked to patient morbidity, elevated rates of drug attrition, and expensive post-marketing withdrawals. The pathogenesis is greatly mediated by hepatic bio-activation and systemic exposure to reactive metabolites, which cannot be effectively studied using conventional in vitro assays and animal models. Such conventional methods usually fail to consider inter-organ crosstalk, which undermines the translation of risk to the kidney and poor results in translation. To fill this gap, we point to the superior liver-kidney organ-on-chip (OoC) platform which accurately represents interactions between hepatic metabolism-renal injury. This dual-tissue, micro-engineered system incorporates liver and kidney cellular analogs on a microfluidic circuit which allows continuous exchange of metabolites and real-time determination of drug disposition, transporter activity and cytotoxicity. The platform uncovered essential injury pathways such as oxidative stress, apoptosis, mitochondrial dysfunction, and biomarker changes as the use of cisplatin and acetaminophen as model nephron-toxicants demonstrated. Mechanistic analysis of injury is normally lost in non-dynamic monocultures or animal models. In addition to predictive toxicology, this technology has a great deal of translational potential. The use of patient-derived cells will facilitate the individual nephrotoxicity profiling, and the combination with the multi-omics and high-content analytics will provide a systems-level view of the organ-organ interactions. It is also human physiologically relevant and reproducible, which is consistent with the current regulatory efforts to reduce animal testing and enhance preclinical predictability. Together, liver-kidney organ-on-chip is a revolutionary technology in mechanistic nephrotoxicity studies, enhances the validity of drug safety testing, speeds safer therapeutic development, and offers the precision medicine solution to renal risk assessment research.

Keywords: Nephrotoxicity, oxidative stress, organ-on-chip, multi-omics etc.

1. Introduction

Drug Induced Nephrotoxicity (DIN) is one of the most daunting issues in drug discovery, translational pharmacology, and regulatory science. Although molecular toxicology and safety pharmacology have improved, DIN remains a major cause of acute kidney injury (AKI) and chronic kidney disease (CKD) in many countries, which causes morbidity, health care expenditures, and drug failure in development[1]. Its development in the late phase clinical trials or post-marketing surveillance is usually associated with dire clinical outcome, regulatory limitation and costly recall. This translational disconnect highlights the constraints of existing preclinical nephrotoxicity testing model and the pressing requirement of physiologically realistic predictive models [2]. The physiological peculiarities of the kidney, such as a high renal plasma flow, expression of transporter in the segments, active tubular secretion and reabsorption, and structural specialization of proximal tubules make the kidney highly susceptible to parent drugs and bioactivated intermediates. Compared to other organs, high local concentrations of drugs that are distributed throughout the body are exposed to the kidney and accumulate in the tubular lumen and parenchyma. DIN has a clinical spectrum ranging between subclinical injury to irreversible renal failure and is mediated mechanistically by direct tubular cytotoxicity, oxidative stress, mitochondrial dysfunction, disturbed hemodynamics, immune-mediated responses and crystal nephropathy [3]. Historically utilized biomarkers, serum creatinine, and blood urea nitrogen (BUN) are not very sensitive to detect early damage and frequently reveal it when most nephrons are already damaged, which does not give them much room to implement therapeutic interventions.

Existing preclinical models have some inherent weaknesses in terms of being able to mimic nephrotoxicity in humans. Two-dimensional (2D) culture of renal epithelial cells do not have tissue polarity, physiological shear stress, and localization of transporters that are essential in drug bioactivity. Organoids and spheroids provide a better tissue structure, however, they are immobilized and frequently do not have perfusable vascularity or active transporter systems [4]. Although animal models are invaluable in systemic exposure research, they are limited due to interspecies expression differences in transporter (e.g. in OAT1/3, OCT2) and metabolism as well as renal clearance. Consequently, they produce low predictive fidelity- safe drugs in animal species may produce nephrotoxicity in humans and vice versa. Moreover, a big fraction of nephrotoxicity is not imposed by the parent drug but by the reactive hepatic metabolic products generated downstream which underscores the critical role of inter-organ crosstalk in renal toxicodynamics [5]. OoCs with liver-kidney organ on chip (OoC) systems are currently redefining preclinical nephrotoxicity assays through the real-time accurate recapitulation of human-relevant hepatic metabolism and hepatic injury. These microengineered systems interface hepatocyte and renal proximal tubule modules via a circuit of microfluidics to allow the dynamic exchange of metabolites, nutrients and signaling molecules [6]. This integration, in contrast to the fixed ones, addresses the dynamics of drug metabolism and transporter activity, and toxic intermediate build-up across time and space. Preliminary experiments on nephrotoxicant models that include cisplatin, tenofovir, and acetaminophen indicate that these models have the potential to recapitulate clinically relevant injury phenotypes that include proximal tubule stress, KIM-1 expression, oxidative stress, and mitochondrial depolarization [7]. More to the point, subclinical toxicities and early stress signatures of OoC have been uncovered and can be treated at pre-injury-stages, which conventional models fail to detect.

New scientific breakthroughs have also improved the fidelity of such systems. The inclusion of vascular endothelial cell and immune cells allows the nephrotoxicity caused by inflammation to be modelled. Clinical injury biomarkers have been revealed by single-cell RNA sequencing and proteomic profiling in coupled liver-kidney chips of early gene-expression perturbations in oxidative stress, Nrf2-activation, and apoptosis pathways. Multi-omics integration with the assistance of AI is currently being used to produce predictive nephrotoxicity signatures that are consistent with actual-world pharmacovigilance data [8]. Also, the renal cells obtained by inducing pluripotency in patients (iPSC)-derived renal cells enable the modelling of inter-individual

susceptibility, such as genetic polymorphism in transporters (e.g., SLC22A2, ABCB1), in detoxifying enzymes (e.g., GSTP1, SOD2), and in inflammatory mediators (e.g., TNF- α). This allows profiling nephrotoxicity of high-risk subgroups of patients and targeted treatment [9]. The OoC technologies are quickly gaining acceptance in regulatory science. The Innovative Science and Technology Approaches to New Drugs (ISTAND) provided by the U.S. FDA and the microphysiological system frameworks proposed by the EMA are in the limelight of these platforms as valid regulatory decision-making tools. Similar initiatives are now in progress to come up with standardized manufacturing, reproducibility measures and assay validation methodologies so that they can be incorporated in safety pharmacology pipelines. Notably, these models provide the possibility of reduction or elimination of animal testing, which is in line with the 3Rs idea worldwide (replacement, reduction, refinement) and enhances human physiological significance [10]. Liver-kidney OoC systems are a paradigm change in the context of high-impact translational research because it allows the evaluation of mechanism-resolved nephrotoxicity at preclinical levels. They offer a bridge to molecular pharmacology and dynamic organ physiology, thereby offering unparalleled resolution of early toxic events, real-time metabolite flux and patient-specific risk signatures. This makes them even more predictive and translational with the addition of AI, systems pharmacology, and computational modeling [11].

2. Drug-Induced Nephrotoxicity: Pathophysiological Basis and Clinical Insights

Nephrotoxicity caused by drugs is one of the most important iatrogenic complications of contemporary medicine as it occupies a significant portion of acute kidney injury (AKI) and leads to the development of chronic kidney disease (CKD) on the global scale. It has a significant effect on morbidity of patients, reduces the therapeutic effects of life saving drugs and burdens healthcare [12]. The physiological peculiarities of the kidney such as high renal perfusion, effective glomerular filtration, active transport along tubules that require energy and specific ability to concentrate xenobiotics in particular render it quite vulnerable to the toxic insults. The renal tissues are exposed to continuous circulation of drugs and its metabolites because about one-quarter of cardiac output is dedicated to the kidneys [13]. Such long term exposure, together with accumulation of xenobiotics within the renal parenchymal, promotes cellular stress, structural damage and progressive renal failure. The proximal tubular epithelial cells (PTECs) are the most damaged cells by drugs among nephron segments. High metabolic demand and expressed drug transporters These cells have high metabolic demands and express multidrug and toxin extrusion proteins (MATEs), organic cation transporters (OCTs) and organic anion transporters (OATs) [14]. Even though these transporters play a vital role in renal clearance, they unintentionally contribute to the accumulation of nephrotoxic compounds intracellularly and worsen the responses to subcellular stress. The surpluses build up results in oxidative stress, mitochondrial dysfunction as well as collapse of energy homeostasis. Stimulated pattern recognition receptors by the release of danger-associated molecular patterns (DAMPs) by damaged PTECs cause innate immune cascades [15]. Continued injury triggers maladaptive repair processes which are typified by tubular atrophy, interstitial fibrosis, and permanent nephron loss.

Oxidative stress and mitochondrial damage initiates the molecular pathogenesis of drug induced nephrotoxicity. Increased reactive oxygen species (ROS) interfere with mitochondrial electron transport, destroy lipids, proteins, and DNA, and depletes ATP, eventually negatively affecting the cellular repair processes [16]. In the moderate level of stress, the release of mitochondrial cytochrome c activates caspase cascades that cause apoptosis, and more severe damage causes necrosis and unregulated leakage of pro-inflammatory mediators, which further increase tissue damage [17]. The inflammation is a significant trigger in disease development. DAMPs of damaged PTECs activate toll-like receptors (TLRs) especially TLR2 and TLR4 which converge on NF- κ B signaling to cause powerful inflammatory cytokines, including TNF- α , IL-1 β , and IL-6. The cytokine environment attracts neutrophils and macrophages, which enhances the production of ROS and tissue damage [18]. Chronic inflammation involves TGF- β /Smad signaling which enhances

deposition of extracellular matrix, fibrosis and structural remodelling. Interaction of oxidative stress and inflammatory signals will develop a feed-forward loop promoting renal injury [19]. The level of drug handling through transporters is one of the critical factors of personal vulnerability. The effect of antivirals and NSAIDs via OAT, cisplatin and aminoglycoside via OCT2, and impeded efflux via MATE raise intracellular toxic load. The intensity of nephrotoxicity is modulated by genetic polymorphisms, epigenetic control and drug-drug interactions that modify the functionality or expression of transporter, which underscores the possibility of nephrotoxicity risk stratification at a personal level [20].

These mechanisms are represented by representative drug classes. Cisplatin which is commonly used in cancer therapy enters PTECs through the OCT2 and triggers mitochondrial dysfunction, oxidative stress, and apoptosis via the p53/MAPK pathway [21]. Cyclosporine A stimulates acute vasoconstriction and chronic fibrosis through the stimulation of TGF- β . Aminoglycosides that are internalized via megalin-cubilin receptors cause lysosomal rupture, the formation of ROS, and necrosis. NSAIDs inhibit the production of prostaglandins, a fact that decreases the renal perfusion and creates a predisposition to ischemia [22]. Antivirals including tenofovir and chemotherapeutics including methotrexate and vancomycin have direct cytotoxic injury and crystal nephropathy. Finally, nephrotoxicity caused by drugs is a complex interaction of oxidative stress, mitochondrial pathology, uncontrolled and programmed cell death, inflammatory exertion, and disposition of drugs via transporters [23]. These converging processes rely on PTECs. The further mechanistic insight will facilitate the discovery of biomarkers earlier, allow precision profiling with the assistance of the transporter, and create specific nephroprotective approaches. Through the combination of systems pharmacology, multi-omics and personalized solutions, renal toxicity can be reduced without compromising therapeutic effects. This accuracy nephroprotection paradigm tackles next-generation translational medicine, which provides an avenue to safer and more effective drug therapies [24].

3. Rationale for Liver–Kidney Coupling in Organ-on-Chip Systems

Nephrotoxicity caused by drugs, is one of the most complex and multi-organ pathophysiological mechanisms, where the impairment of renal functions is usually not caused by the parent drug, but rather by the hepatically produced metabolites. The traditional *in vitro* models and *in vivo* studies with animals often do not include this complex interaction of hepatic bioactivation and renal exposure, which leads to partial or incorrect toxicity profile [25]. To overcome this essential translational gap, the combination of liver and kidney organ functions in a microengineered organ-on-chip (OoC) platform has become a revolutionary technology. This model is a two-organ system which allows modeling metabolic activation, systemic circulation and renal exposure in a manner that closely resembles human physiology [26]. This is not only an effective way of improving mechanistic knowledge of nephrotoxicity but is also much better than enhancing preclinical risk assessment and drug safety assessment. The dual-organ technique is critical since a number of clinically significant nephrotoxicants can only cause their toxicity through hepatic metabolism activation. Such compounds might not be detected by conventional renal monocultures, which do not express phase I and II metabolic enzyme activity and cause late-onset nephrotoxic liabilities to be detected in late-phase clinical trials or in post-marketing surveillance [27]. This limitation is addressed by a liver-kidney coupled OoC platform where both the parent compounds and the metabolites are exposed to renal proximal tubular epithelial cells after the sequential liver biotransformation of drugs in a hepatic module. This biologically applicable model with great fidelity to *in vivo* pharmacodynamics and pharmacokinetics allows detection of early renal safety risk [28].

The liver has a key metabolic activation role, with lipophilic drugs going through phase I biotransformation involving the activity of cytochrome P450 (CYP) isoforms and phase II conjugation processes like glucuronidation, sulfation and acetylation. Although these processes tend to produce hydrophilic, excretable products, they may produce reactive intermediates which covalently bind nucleic acids, proteins and lipids and induce the oxidative stress and the activation

of immune responses[29]. These hepatically produced reactive metabolites then to be released into the circulation and are taken to the kidneys in large quantities as the organ has a high perfusion and filtration rate. The renal uptake, as well as intracellular accumulation, relies on the presence of transporters like organic anion transporters (OATs), organic cation transporters (OCTs), and multidrug and toxin extrusion proteins (MATEs). Also, the production of hepatically produced inflammatory mediators may enhance the extent of renal injury by priming immunity and modifying the renal hemodynamics, establishing a multi-organ-damaging pattern [30]. These interactions are clinically relevant as demonstrated by the use of case studies. The hepatotoxicity classical acetaminophen also produces nephrotoxicity through the action of the reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which mediates oxidative stress and fermentation in proximal tubular cells. Nephrotoxic derivatives of tenofovir produced in the liver accumulate due to uptake by OAT resulting in the Fanconi syndrome [31].

On the same note, NSAIDs produce reactive intermediates that inhibit the production of prostaglandins, triggering ischemic kidney damage. The chloroacetaldehyde and acrolein, which are the products of ifosfamide and cyclophosphamide respectively, are strong nephrotoxic metabolites which cause dose-limiting toxicities. Using hepatic and renal tissue analogs on OoC platforms on microfluidics, the researchers will be able to recap the real-time inter-organ communication, metabolic activation, transporter-mediated uptake, and injury mechanisms under physiologically relevant flow conditions [32]. It can also be further personalized by the use of patient-derived cells or induced pluripotent stem cell (iPSC)-derived organoids, which can be used to measure toxicity and apply precision medicine.

4. Principles of Organ-on-Chip Technology

One of the most critical physiological interfaces governing drug metabolism, detoxification, and elimination is the liver-kidney axis, a cornerstone of contemporary pre-clinical safety assessment. The advent of liver-kidney organ-on-chip (OoC) systems represents a paradigm shift in toxicology and pharmacology by providing an integrated, mechanistically detailed assessment of drug-induced nephrotoxicity that surpasses the predictive capacity of conventional in-vitro and animal models [33]. These coupled microphysiological devices are engineered to recapitulate the bidirectional crosstalk between hepatic and renal compartments, thereby overcoming the limitations inherent to isolated organ systems. Early OoC platforms largely focused on single-organ modules, enabling the study of hepatic metabolism or hepatic toxicity in isolation. While informative, such compartmentalized approaches fail to capture metabolite-mediated nephrotoxicity a prevalent form of drug-induced injury in humans [34]. Nephrotoxic liabilities arise not only from parent compounds but also from reactive metabolites generated during hepatic bioactivation. These metabolites, once delivered to the kidney, inflict damage on proximal tubular epithelial cells via oxidative stress, mitochondrial dysfunction, and apoptosis—an ordered interaction unattainable within isolated organ cultures. Liver-kidney interconnected platforms bridge this gap by establishing a physiologically relevant circuit that embodies systemic drug metabolism, distribution, and clearance.

Architecturally, liver-kidney OoCs incorporate microfluidic circuits that link hepatic and renal tissue chambers. Two predominant configurations exist: sequential flow systems, which mimic natural circulation by perfusing the hepatic module first and then the renal module; and parallel flow systems, which allow simultaneous perfusion of both compartments and enable the dissection of direct drug effects on each organ without metabolic confounding. Sequential models are particularly valuable for evaluating metabolite-driven toxicants such as cisplatin or acetaminophen, whereas parallel systems afford greater flexibility in probing direct organ-specific actions of candidate drugs. Tissue architecture within each module is of paramount importance. Polarity of cells, extracellular matrix composition, and multicellular organization are poorly represented in traditional two-dimensional monolayers [35]. Three-dimensional constructs—spheroids, scaffold assemblies, and gel-based cultures—better recapitulate cell-cell and cell-matrix interactions, preserve functional phenotypes, and extend culture stability. In the hepatic module, the inclusion of

parenchymal hepatocytes alongside non-parenchymal cells (Kupffer cells, hepatic stellate cells, and sinusoidal endothelial cells) recapitulates lobular organization and enhances metabolic fidelity [36]. The renal module frequently co-cultures proximal tubular epithelial cells with endothelial and interstitial cells, thereby mimicking nephron-like architecture and polarized transporter expression that govern drug uptake and efflux. Fabrication techniques leverage advanced microengineering. While polydimethylsiloxane (PDMS) remains popular for its optical transparency and rapid prototyping, its drug absorption and limited long-term stability have spurred the adoption of thermoplastics, hybrid polymers, and photo-reactive hydrogels [37]. Micromilling, laser ablation, and lithographic multilayering enable increasingly complex channel geometries and vascular-like structures. Bio-printing has recently empowered the spatial patterning of heterogeneous cell populations with precise resolution, facilitating zonal hepatocyte heterogeneity or segmented nephron architecture [38]. When coupled with microfluidic perfusion, bio-printing yields highly physiological processes, enabling robust modeling of inter-organ interactions and the creation of patient-specific scaffolds from induced pluripotent stem cell (iPSC)-derived cells. Dynamic perfusion is a defining feature of these systems, ensuring continuous delivery of nutrients and oxygen while removing waste products and exposing cells to physiologically relevant shear stress [39-40]. Continuous flow not only preserves cell viability but also propagates mechanosensitive signaling pathways that regulate metabolism, differentiation, and injury responses. The integration of embedded biosensors allows real-time monitoring of pH, oxygen gradients, metabolite flux, and biomarker release, yielding sustained and clinically relevant readouts of drug effects [41-42].

So that, The liver-kidney OoC platforms represent a transformative tool for predictive toxicology, faithfully recapitulating the physiological, cellular, and biochemical complexity of the liver-kidney axis. Their adoption promises to refine drug safety assessment, reduce reliance on animal studies, and accelerate the development of safer therapeutics [43-44]. Together, liver-kidney OoC systems represent the intersection of microfluidics, biomaterials and tissue engineering to recapitulate human drug metabolism and renal toxicity like never before. These systems will transform preclinical pipelines by enabling the mechanistic investigation of metabolite-mediated nephrotoxicity, the identification of biomarkers, and inter-organ pharmacokinetics [45]. Their sophistication and regulatory approval will be crucial to reduce animal utilization, enhance the translational precision and develop the next generation of precise toxicology and customized therapeutics [46].

5. Liver–Kidney Organ-on-Chip Systems: Design and Architecture

The future of the translational pharmacology and toxicology is the integration of hepatic and renal microphysiology on a single microfluidic platform. A set of principles in design to achieve maximum physiological relevance with a minimum of engineering tractability has coalesced around three broad concepts: (i) Cellular compartmentalization of reflecting in-situ tissue heterogeneity (ii) fluidic coupling of maintaining unidirectional metabolic flux, (iii) multi-analyte sensing of providing real-time functional readouts[47-48].

Primary hepatocytes or hepatocytes of the induced-pluripotent-stem-cell-based hepatocytes are then seeded in a collagen- or Matrigel-based matrix to recap the sinuoidal microarchitecture in the cellular compartment. Parallel to it, proximal tubule epithelial cells (PTECs) proliferate on a porous polyester or polycarbonate membrane, which is equivalent to the glomerular filtrate-to-tubule gradient [49-50]. This interface of the hepatocyte channel with the PTEC channel is designed by a micro-fabricated ductus which provides uninterrupted and laminar flow of medium so that metabolites produced by hepatic CYP450 enzymes are transported to renal epithelium at physiologically important concentrations. Of particular importance to this design is the application of gravity-balanced flow by either peristaltic or syringe-pump operation, maintained at shear stresses of 0.1-0.3-1-cm-1, the level that maintains hepatocyte polarity as well as PTEC transporter activity [51-53].

Fluidic coupling is obtained as a product of a succession of serpentine channels and micro-cavities which subject the directional flow which is akin to in-body pathway of liver-to-kidney.

Computational fluid dynamics (CFD) is applied to optimise the channel geometry to provide a residence time of 1520 min to the hepatocyte-derived metabolites to get to the kidney module [54-55]. This time window is necessary in the faithful simulation of first pass metabolism. Below the kidney module, a waste collection chamber, with a nanofiltration unit, is provided to enable the excretory products to be collected which can then be downstream to mass spectrometry or LC-MS/MS analysis without pre-processing of the sample [56]. A layered architecture of sensors and optically transparent windows is combined to provide real-time functional readouts. One of the electrochemical impedance spectroscopy (EIS) probes is inserted into the hepatocyte channel and the receptor of the kidney is a micro-fluidic biosensor where creatinine clearance and glomerular filtration rate (GFR) are measured via fluorescence-based assays [57]. The integration of a micro-electrode array (MEA) allows measuring bilirubin conjugation and urea production metabolic fluxes that would result in a multiplexed readout, much higher than the traditional one-point assays would yield [58-59]. It has a general architecture of a modular system that can be scaled in parallel to investigate combinatorial drug-drug interactions or disease conditions, including hepatic steatosis and hepatic nephrotoxicity. The system is also compatible with the co-culture of non-parenchymal cells such as Kupffer cells and hepatic stellate cell to recreate immune-mediated or fibrotic response [60-61]. This organ-on-chip system is a high-throughput, reductionist platform capable of predicting systemic pharmacokinetics, detecting off-target toxicity, and facilitating the discovery of safer therapeutics by bridging liver and kidney in a physiologically relevant, continuously perfused microenvironment [62-63].

6. Cellular and Molecular Metrics for Evaluating Nephrotoxicity Using Liver–Kidney Organ-on-Chip Platforms

Drug induced nephrotoxicity (DIN) has been one of the most recalcitrant challenges in the area of drug development, leading to late-stage clinical failure and in most instances, premature discontinuation of promising therapeutics. The conventional mono-culture in-vitro monolayers, kidney organoids and animal models are not sufficient since they lack the capability to recapitulate the complex architecture and segment-differentiated networks of transporters of the human kidney as well as the critical role played by the hepatic metabolism in the formation of nephrotoxic metabolites [64-66]. The emerging technology of liver-kidney organ-on-chip (OoC) technology has closed this translational divide and provided a human-relevant, mechanistic, and multi-scale assessment platform which recapitulates the physiological hepato-renal interaction in real time. The core of this system is a microfluidic body-in-a-chip that interacts with primary hepatocytes or IPS cells derived liver cells with proximal tubular epithelial cells (PTECs) [66-68]. The forced circulation created permits uninterrupted perfusion, which creates physiologic shear forces which do not exist in static culture. This flowing system allows pulsatile drug dosing, real-time transfer, and longitudinal tissue response to be monitored and, therefore, the initial, subclinical events that can take place before overt tissue nephron loss can be detected. Traditional biomarkers e.g. serum creatinine or BUN increase when the tubules have undergone significant disruption; OoC platforms, on the other hand, can monitor subtle functional disruption before clinical by-products are violated [69-70].

Cellular and Molecular Metrics for Evaluating Nephrotoxicity Using Liver–Kidney Organ-on-Chipforms

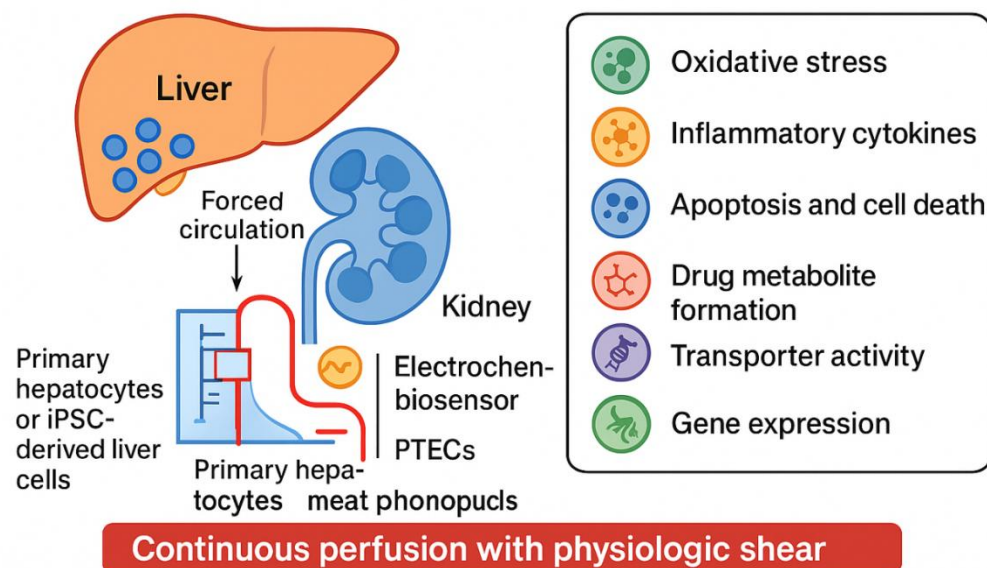


Figure 1: Integrative liver–kidney organ-on-chip platform illustrating cellular and molecular metrics for nephrotoxicity assessment. Continuous perfusion enables hepatic metabolism, metabolite transfer, and renal response monitoring through embedded biosensors that track oxidative stress, apoptosis, and injury biomarkers in real time.

Sentinel role of PTECs is supported by the fact that they are highly metabolic, with dense repertoire of transporters and vulnerable to segments. Up-regulation of secreted proteins that are signals of dedifferentiation and regenerative efforts (Kidney Injury Molecule-1 (KIM -1), transmembrane glycoprotein) and rapid oxidative stress/hypoxia responses (Neutrophil Gelatinase -Associated Lipocalin (NGAL)) is an early warning of injury [71-72]. The chaperone-like protein, clusterin, also provides information on the dynamics of tissue remodeling. Such biomarkers can be measured in the chip effluent by the conventional ELISAs or more conveniently in real time with embedded electrochemical biosensors and can offer non-invasive, longitudinal monitoring of the health of the kidneys. It is also very important that a hepatic compartment is included, which accurately recreates drug biotransformation. Nephrotoxicants, including cisplatin, acetaminophen, aristolochic acid, etc., are reactive or toxic when converted by the hepatic cytochrome P450 (e.g., CYP3A4, CYP2C9, CYP2D6) [73-75]. Tracking albumin secretion, urea production, and metabolite levels, right after investigating active hepatic metabolic activity enables researchers to verify active hepatic metabolism and to make sure that the kidney exposure recapitulates the realistic in-vivo pharmacokinetics. This combined metabolism-to-nephrotoxicity interface provides clinically useful toxicity information that would otherwise have been obtained through trials in their advanced stages [76-77].

OoC systems accept multi-omics integration to enhance the understanding of mechanistic insight. Transcriptomics identifies premature induction of oxidative stress signaling (Nrf2), inflammatory (NF- κ B) and apoptotic (caspases) modules [78]. The proteomics indicates dynamic alterations in the expression of transporters, cytoskeletal assembly and activity, and the metabolomics shows alterations in the energy metabolism, amino-acid metabolism and lipid metabolism. Combining these data sets produces a systems level fingerprint of nephrotoxic progression and allows injury pathways to be dissected accurately and point of therapeutic intervention to be identified [79-80]. Overall, liver-kidney organ-on-chip systems are a paradigm shift in the evaluation of nephrotoxicity and they have unmatched physiological relevance, real-time, and mechanistic complexity [81-82]. Their implementation would result in a significant decrease in the rates of attrition, shortening of

drug development cycles, and ultimately, avoiding unintentional damage to the kidneys of patients [83].

Table 1. Comparative analysis of Liver–Kidney OoC vs conventional nephrotoxicity models [84-90].

Feature / Attribute	2-D Static Cultures	Cell Animal Models	Liver–Kidney OoC Platforms
1 Cellular complexity	Simple monolayers, limited cell types	Whole-organ complexity but species-specific	Multi-cell co-cultures (hepatocytes, tubular cells, endothelium, immune cells)
2 Tissue architecture	Flat, non-polarized	Native 3D organ anatomy (species differences)	3D or polarized microtissues with physiological geometry
3 Cell polarity	Poorly maintained	Preserved in vivo	Preserved via ECM, flow and architecture
4 Transporter expression	Often low or mislocalized	Native expression but species-dependent	Physiological expression and localization (OATs, OCTs, P-gp)
5 Drug-metabolizing enzymes	Limited CYP activity	Robust but species-specific CYP profiles	Human-relevant CYP expression and inducibility
6 Hepatic bio-activation modeling	Absent or artificial	Present but may differ from humans	On-chip hepatic metabolism feeding renal compartment
7 Perfusion / fluid dynamics	Static exposures	Systemic circulation, uncontrolled shear	Controlled microfluidic flow reproducing shear stress
8 Dynamic dosing / PK mimicry	Single dose or repeated static dosing	Physiological PK but species difference	Recreates concentration–time profiles and clearance
9 Inter-organ crosstalk	Absent	Present in whole animal	Direct fluidic coupling for metabolite transfer
10 Immune component modeling	Minimal (if included)	Full immune system (complex)	Tunable inclusion of immune cells for inflammation studies
11 Vascularization	Lacking	Native vasculature	Endothelialized channels mimicking microvasculature
12 Long-term culture stability	Limited (days)	Long term in vivo	Sustained culture (weeks) with maintained function
13 Throughput	High (simple, plate format)	Low to moderate	Moderate — improving with parallelization and automation
14 Scalability for HTS	Readily scalable	Not scalable	Scalable with design standardization and robotics
15 Reproducibility	High within lab; cell-line dependent	Variable between species and centers	Improving — depends on SOPs, materials, cell source
16 Standardization	Simple assays, easy SOPs	Hard to standardize across species	Requires harmonized protocols and benchmarks
17 Species relevance to humans	Human cell lines possible but immature	Often poor translational predictivity	High human relevance when using human cells
18 Ability to model metabolite-driven toxicity	Poor	Possible but species differences limit relevance	Excellent — liver produces human metabolites delivered to kidney
19 Biomarker correlation with clinic	Limited (few secreted markers)	Variable correlation	Strong correlation (KIM-1, NGAL, cystatin C) with real-time effluent sampling
20 Real-time monitoring capability	Limited (assays) (endpoint)	Some telemetry in vivo	Integrated biosensors (O ₂ , pH, impedance, biomarkers) for continuous readout
21 Sampling accessibility	Easy (supernatants, lysates)	Invasive or terminal for tissue	Frequent, non-destructive effluent sampling ports
22 Imaging and visualization	Compatible microscopy	In vivo imaging limited resolution	Optical transparency and high-resolution live imaging
23 Measurement of cellular energetics	Indirect assays	In vivo measures exist but complex	Direct measures (OCR, mitochondrial potential) via sensors
24 Cost per experiment	Low per assay	High (animal housing, ethics)	Moderate–high initially; decreases with scale and reuse
25 Ethical considerations	Minimal	Significant animal welfare concerns	Ethically favorable (reduces animal use)
26 Regulatory acceptance	Established assays exist	Widely used historically	Emerging; qualification in progress with agencies

	Feature / Attribute	2-D Static Cultures	Cell Animal Models	Liver–Kidney OoC Platforms
27	Data richness (multi-omics)	Limited scope	Possible but complex and costly	Highly amenable to integrated multi-omics (transcriptome, proteome, metabolome)
28	Integration with PBPK / in silico models	Simple inputs only	Useful data but species gaps	Provides human-relevant parameters for PBPK and AI models
29	Patient-specific modeling	Possible with primary cells but limited	Possible with humanized models; costly	Readily supports iPSC or patient-derived cells for personalized predictions
30	Time to actionable insight in R&D	Rapid screening but low predictivity	Slower, costly, translational uncertainty	Mechanistic insights earlier with higher translational value

7. Mechanistic Evaluation of Drug-Induced Nephrotoxicity Using Liver–Kidney Chips

The liver-kidney organ-on-chip (OoC) platform is quickly transforming the pre-clinical nephrotoxicity method. The system simulates the bidirectional recreational of metabolites exchange between hepatocytes and proximal -tubule cells of the kidney through the curvatures of microfluidics to create the microfluidic network. This dynamic architecture subjects physiological shear stress, pulsatile flow, and nutrient gradients which mimic the environment of the human circulatory system as opposed to the conventional simple cultures or rodent models [91-92]. This means that the processes of drug metabolism and excretion should be tracked in real-time, providing a physiologically honest outcome of both hepatic clearance and renal clearance.

The major strength of the OoC is the possibility to record metabolite-dependent nephrotoxicity which has been historically underestimated. Metabolic biotransformation in the liver module at the phase I and phase II is mediated by CYP3A4, CYP2C9, CYP2D6 and conjugating enzymes which form reactive intermediates. The metabolites are then transported into the kidney chamber by clinically relevant transporters like OAT1/3, OCT2, and P -glycoprotein. The metabolites may initiate oxidative stress, mitochondrial dysfunction, apoptosis, necroptosis, or inflammatory cascades once within the cells of the proximal-tubule [93-94]. Observing those pathways in vivo, the researchers will be able to subdivide the mechanisms of injury onset and determine treatment targets with more accuracy.

The most useful application of the platform is most demonstrated by its use in a variety of nephrotoxic agents. Indicatively, cisplatin is initially conjugated with glutathione in the liver, to form reactive thiol species that build up in the kidney and cause the production of reactive oxygen species, mitochondrial swelling and caspase-mediated cell death [95]. By reproducing the acute kidney injury clinically, methotrexate toxicity is simulated by recreating crystal formation in the intratubules and urine flow [96]. The immunosuppressants such as cyclosporine A and tacrolimus which are metabolized complexly by the liver are demonstrated to cause tubular vacuolization, oxidative damage, and induce pro-fibrotic gene expression of TGF- β . Among the antibiotics, too, including aminoglycoside drugs, e.g. gentamicin, are faithfully modelled; the chip recreates megalin-cubilin-mediated endocytosis, chemistry researchers are able to measure drug concentration, establish exposure limits, and monitor early biomarkers like KIM -1 and NGAL shown in figure 2 [97].

In addition to pharmaceuticals, the liver -kidney OoC is also invaluable in studying the environmental and botanical nephrotoxins. The classical herbal toxin is Aristolochic acid that is activated hepatically to create adducts of DNA that spread interstitial fibrosis and renal carcinogenesis. This human specific sensitivity in predicting these adverse outcomes with the chip is even more effective than the traditional methods in that it minimizes the use of animals and shortens the time it takes to profile the safety of drugs [98]. Overall, the liver-kidney organ-on-chip provides a more human, multifaceted platform that reveals drug metabolism, kidney transport, and injury signaling complexity [99]. It is an invaluable instrument in drug development, which occurs easier and more precisely in transplantation, translational relevance, and the measurement of synthetic and natural nephrotoxin because of its real-time monitoring, mechanistic depth, and translational relevance [100].

Liver-kidney organ-on-chip (OoC) devices transform pre-clinical testing of nephrotoxicity by connecting hepatic drugs metabolism and renal excretion in a single and human organ micro-physiological system. Human hepatocytes and kidney tubular epithelial cells are in interconnected microfluidic chambers which recreate systemic circulation, shear stress, and nutrient exchange- far more than can be achieved by the stagnant cultures or animal models [101]. The design allows real-time and continuous observations of drug metabolism, the transfer of metabolites, and tissue-specific injury, which provides a human-relevant mechanistic perspective of drug-induced kidney injury [102]. The chips are also good in the model of metabolite-dependent nephrotoxicity, which traditional assays usually overlook. Phase I/II liver module metabolism (via CYP3A4, CYP2C9, CYP2D6) produces bioactive intermediates which are circulated in the blood to the kidney through transporters, including OAT1/3, OCT2, and P-glycoprotein. These metabolites are then exposed to proximal tubular cells, where the presence of oxidative stress, mitochondrial dysfunction, apoptosis, necroptosis, and inflammation that culminate in early injury and emphasize therapeutic targets can be determined [103]. The platform is particularly useful with nephrotoxic chemotherapeutics: cisplatin, when hepatic glutathione conjugated, forms reactive species that induce ROS, mitochondrial swelling, and caspase-mediated apoptosis; methotrexate-induced AKI is recapitulated by modeling the urine flow and crystal precipitation; cyclosporine A and tacrolimus create metabolites that trigger tubular vacuolisation, oxidative damage, and TGF- β -stimulated fibrosis. Dose can be optimised in transplant patients using chronic mechanisms [104].

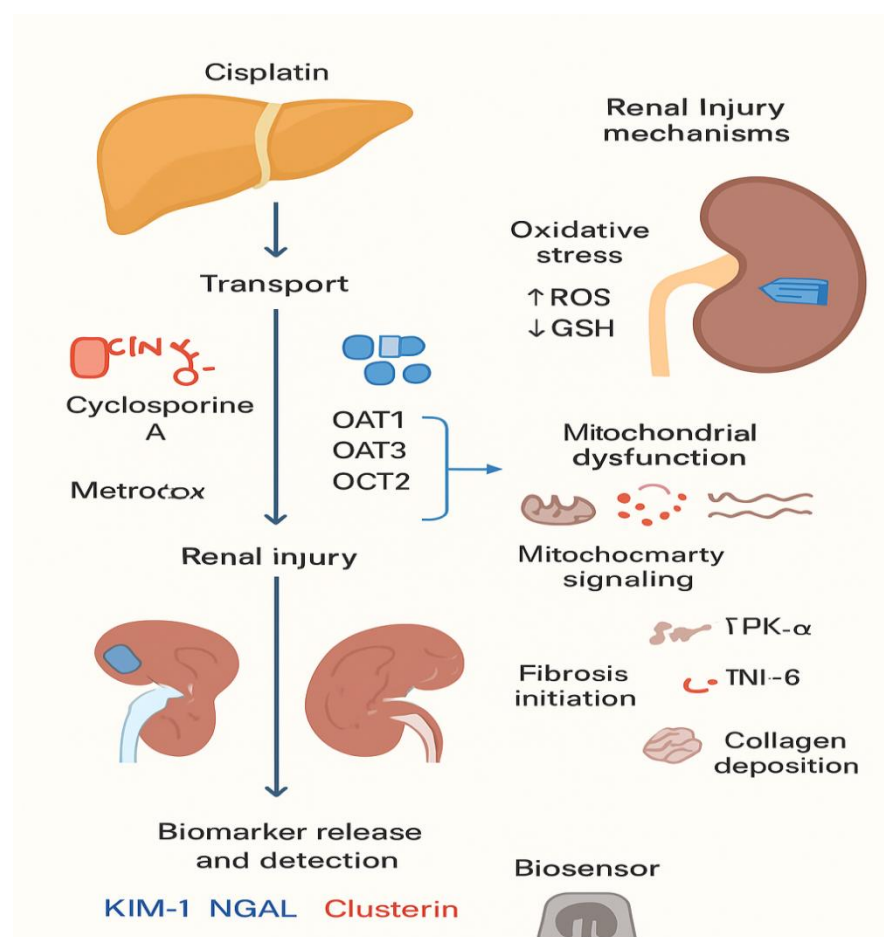


Figure 2: Schematic representation of drug-induced nephrotoxicity on a liver–kidney organ-on-chip. Drugs undergo hepatic bioactivation, are transported via OAT1, OAT3, and OCT2, triggering renal oxidative stress, mitochondrial dysfunction, inflammation, and fibrosis. Injury biomarkers (KIM-1, NGAL, clusterin) released into microfluidic effluent enable real-time toxicity detection and mechanistic evaluation.

The endocytosis of gentamicin through megalin/cubilin is recapitulated which allows determining the accumulation thresholds and the early biomarkers KIM -1 and NGAL to be determined (Table 2) [105]. In addition to drugs, environmental and herbal nephrotoxins including Aristolochic acid have been examined using the liver-kidney OoC in which DNA adducts were observed and interstitial fibrosis was detected. This platform enhances drug discovery, safety testing, and personalised nephrotoxicity treatment by predicting clinical outcomes, decreasing animal models, providing early, sensitive, human-like data [106].

Table 2: Nephrotoxicity Biomarkers in Organ-on-Chip Readouts [107-114]

Biomarker	Primary Source	Biological Significance	Application in OoC
KIM-1 (Kidney Injury Molecule-1)	Proximal tubular epithelial cells	Early indicator of proximal tubular damage	Acute kidney injury detection
NGAL (Neutrophil Gelatinase-Associated Lipocalin)	Tubular cells, collecting ducts	Rapid stress response during AKI	Early marker nephrotoxicity
Clusterin	Tubular and glomerular epithelial cells	Marker of repair and regeneration after injury	Recovery and repair monitoring
Cystatin C	Glomerular filtration	Sensitive marker of early glomerular injury	Chronic kidney injury assessment
Beta-2 Microglobulin	Glomerular and tubular function	Filtered by glomerulus, reabsorbed in tubules	Tubular dysfunction readout
Osteopontin (OPN)	Tubular epithelial cells, macrophages	Marker of inflammation and fibrosis in kidney tissue	Chronic nephropathy modeling
IL-18	Tubular epithelial cells, immune cells	Inflammatory cytokine elevated in drug-induced kidney injury	Inflammatory injury detection
TNF-α	Macrophages, tubular epithelial cells	Central mediator of inflammatory pathways	Cytokine signaling monitoring
IL-6	Tubular and hepatic cells	Systemic inflammatory response marker	Inflammation and stress readout
ROS (Reactive Oxygen Species)	Mitochondria, cytoplasm	Oxidative stress and mitochondrial dysfunction	Mechanistic insight for oxidative damage
GSH (Glutathione)	Cytoplasmic antioxidant system	Cellular redox balance and detoxification	Oxidative stress and drug metabolism
MDA (Malondialdehyde)	Lipid membranes	Lipid peroxidation marker for oxidative injury	Oxidative stress evaluation
NAG (N-acetyl-β-D-glucosaminidase)	Proximal tubular lysosomes	Tubular cell lysosomal integrity marker	Early nephrotoxic response
L-FABP (Liver-type Fatty Acid Binding Protein)	Proximal tubular epithelial cells	Indicator of tubular ischemia and hypoxia	Ischemic injury assessment
CYP3A4 activity	Hepatocytes	Major drug-metabolizing enzyme	Hepatic bioactivation evaluation
CYP2E1 activity	Hepatocytes	Bioactivation of nephrotoxic metabolites	Mechanistic study of reactive metabolites
Albumin secretion	Hepatocytes	Marker of liver synthetic function	Hepatic health monitoring
Urea production	Hepatocytes	Nitrogen detoxification capacity	Metabolic function evaluation
Creatinine clearance surrogate	Systemic flow between liver and kidney modules	Simulation of glomerular filtration rate (GFR)	Functional kidney performance
Metabolite profiles (LC-MS)	Chip effluent	Global drug metabolism and clearance patterns	Pharmacokinetic modeling
HMGB1 (High Mobility Group Box 1)	Injured tubular epithelial cells	DAMP molecule indicating necrosis and inflammation	Necrotic cell death assessment
Caspase-3 activity	Tubular and hepatic cells	Key executioner of apoptosis	Apoptotic injury pathway
LDH (Lactate Dehydrogenase)	Cytosol	General cytotoxicity marker	Overall cell viability check
ATP levels	Mitochondria	Energy metabolism and mitochondrial function	Cellular viability and stress
pH and Oxygen sensors (real-time)	Perfusate, microchannels	Dynamic monitoring of physiological stability	Continuous functional monitoring

8. Integration of Advanced Technologies in Liver–Kidney Organ-on-Chip Systems

The combination of high-resolution analytical systems, multi-cell co-cultures, and more sophisticated microfluidics has brought a breakthrough in the research of nephrotoxicity and liver on chip systems through multi-cellular co-cultures. These next generation systems can recapitulate hepatic drug metabolism, bioactivated metabolites and renal excretion in a manner that has never been possible and creates a solid simulation of inter-organ interactions [115]. Predictive power can be increased with the help of artificial intelligence (AI) and machine learning (ML) that combine real-time outputs of biosensors, imaging streams, and multi-omics data. Algorithms identify the early and minor changes in nephrotoxicity biomarkers, including Kidney Injury Molecule 1 (KIM1), Neutrophil Gelatinase-Associated Lipocalin (NGAL) and clusterin, prior to the death of the organ. AI-powered models also combine chip data and clinical pharmacokinetic/pharmacodynamic datasets, which enables virtual drug screening that can speed up the screening of candidates at lower costs [116].

At the molecular level, CRISPR/Cas9 gene editing produces the hepatocyte and renal cell lines which isolate the functions of transporter (OATs, OCTs) and cytochrome P450 enzymes. This would enable the prediction of nephrotoxicity specific to a patient that is coupled with patient-derived induced pluripotent stem cells (iPSCs) harboring clinically relevant polymorphisms, enabling personalized predictions of nephrotoxicity to consider genetic variations in drug metabolism and vulnerability to injury [117]. The use of endothelial cell-lined vascularized microchannels recreates physiologic perfusion and shear stress, and the combined immune components, including macrophages, neutrophils, lymphocytes, recreates the injury model of inflammatory mechanisms. Cytokines, like TNF - α , IL-6, and MCP-1, increase oxidative stress and tubular injury in cisplatin exposure, which has now been recapitulated in OoC shown in table 3 [118].

Lastly, the systemic injury map can be provided by multi-omics (transcriptomics, proteomics, metabolomics, epigenomics) data between liver and kidney compartments. In combination with bioinformatics and network modeling, these data reveal the presence of new biomarkers, drug targets and personal treatment plans [119]. The combination of AI, gene editing, vascular/immune integration and systems-level omics takes liver-kidney OoCs to the next level of predictive, mechanistic and translational nephrotoxicity measurements [120].

Table 3. Applications of Liver–Kidney OoC Systems Across Drug Development Stages [121–135]

Stage of Drug Development	Specific Application	Mechanistic Focus	Key Readouts / Biomarkers	Advantages Over Conventional Models
Early Discovery	High-throughput screening for nephrotoxicity	Drug-induced cytotoxicity in hepatocytes & renal cells	Cell viability, LDH release, ATP content	Rapid screening of chemical libraries with human-relevant data
	Detection of sub-lethal mitochondrial dysfunction	Energy dysregulation in tubular cells	Mitochondrial membrane potential, OCR	Early detection of non-apoptotic damage
	Identification of reactive metabolite formation	CYP-mediated metabolism	GSH depletion, metabolite profiling	Detects toxic intermediates missed by static assays
	Off-target transporter inhibition screening	OATs, OCTs, P-gp transporters	Transporter activity assays	Mechanistic insight into drug-drug interactions
	Selection of lead candidates with safe profiles	Comparative nephrotoxicity risk	KIM-1, NGAL secretion	Improves lead optimization success rate
Hit-to-Lead Optimization	Dose-response relationship mapping	Progressive injury pathways	Apoptosis vs necrosis ratios	Defines safe therapeutic window
	Evaluation of drug stability and metabolism	Hepatic Phase I & II metabolism	Parent drug clearance rates	Predicts in vivo half-life and stability

Stage of Drug Development	Specific Application	Mechanistic Focus	Key Biomarkers	Readouts	Advantages Over Conventional Models
Preclinical Development	bioactivation				
	Mechanistic evaluation of drug clearance	Tubular secretion and reabsorption	Creatinine, cystatin C		Human-relevant clearance prediction
	Evaluation of efflux pump induction	MDR1/P-gp activity modulation	Real-time transport data		Identifies resistance or toxicity drivers
	Cross-species comparative toxicity studies	Human vs rodent cellular response	Biomarker panels		Improves translation and reduces animal use
	Hepatic-renal PK/PD simulation	Drug absorption and clearance	Time-course metabolite analysis		Mimics systemic pharmacokinetics
	Prediction of metabolite-driven nephrotoxicity	Bioactivation of nephrotoxic species	LC-MS metabolite signatures		Detects toxicity before clinical stage
Toxicology Testing	Modeling inflammatory nephropathy	TNF- α , IL-6 mediated inflammation	Cytokine panels, MCP-1		Replicates immune-driven kidney injury
	Immune-on-chip toxicity testing	Immune-tubular crosstalk	T-cell activation markers		Captures immune-driven drug responses
	Sex-specific toxicity evaluation	Hormonal influence on toxicity	Estrogen/testosterone impact		Personalized gender-based prediction
	Organ perfusion stress modeling	Flow-related shear injury	Endothelial production	NO	Simulates hemodynamic drug effects
	Chronic low-dose nephrotoxicity testing	Repeated drug exposure	Tubular integrity over weeks		Enables long-term monitoring
	Biomarker qualification studies	Validation of novel clinical biomarkers	KIM-1, Osteopontin	Clusterin	Aligns with regulatory biomarker frameworks
Clinical Translation	Drug-drug interaction studies	CYP induction & transporter competition	CYP3A4, OCT2 assays		Identifies potential polypharmacy risks
	Dose-escalation toxicity modeling	Threshold effects identification	ALT, AST, GGT, urea		Predicts first-in-human safe dose
	Modeling cisplatin-induced nephrotoxicity	DNA damage, oxidative stress	8-OHdG, γ -H2AX, ROS		Replicates clinical chemotherapy injury
	Antibiotic-induced renal toxicity	Aminoglycoside accumulation pathways	Gentamicin uptake, lysosomal rupture		Mechanistic mapping of antibiotic nephropathy
	Patient-specific response prediction	iPSC-derived patient cells	Personalized biomarker signatures		Enables individualized therapy planning
	Genotype-phenotype toxicity correlation	Drug-transporter gene variants	SNP-associated panels		Precision medicine-based risk assessment
Regulatory & Post-Marketing	Simulation of rare disease states	Genetic kidney disorders	Cystinosis, pathways	PKD1	Expands translational research applications
	Predictive modeling for vulnerable populations	Pediatrics, geriatrics	Age-specific rates	metabolic	Safer dosing guidelines
	Identification of early preclinical signals	Pre-symptomatic toxicity biomarkers	Omics (transcriptomic shifts)	profiles	Proactive intervention before clinical manifestation
	Adaptive trial design support	Real-time drug response monitoring	Time-course simulation	PK/PD	Improves clinical trial safety and efficiency
	Qualification for FDA/EMA submissions	Context-of-use validation	KIM-1, alignment guidelines	NGAL with	Supports regulatory acceptance

Stage of Drug Development	Specific Application	Mechanistic Focus	Key Biomarkers	Readouts	/ Advantages Over Conventional Models
Advanced Research	Comparative performance vs animal models	Inter-lab reproducibility testing	Cross-platform validation data		Establishes credibility
	Safety monitoring of post-market drugs	Real-world effect replication	Chronic toxicity markers	exposure	Provides ongoing safety surveillance
	Replacement of animal models (3Rs)	Ethical compliance	Reduction of animal use data		Aligns with international ethical guidelines
	Post-approval label refinement	Data-driven adjustments	Real-time OoC	PK/PD	Improves post-market drug safety profiles
	Multi-organ interaction mapping	Liver-heart-kidney effects	Cardiac creatinine, ALT	troponin,	Holistic systemic toxicity profiling
	Modeling drug-induced vascular injury	Endothelial pathways	ICAM-1, VCAM-1		Mechanistic toxicity insights
	Integration with AI for predictive modeling	Deep learning-based toxicity forecasts	Omics, biosensor datasets	imaging,	Accelerates prediction and decision-making
Future Innovations	Incorporation of CRISPR/Cas9 edited models	Gene-function validation	Transporter in lines	KO/knock-	High precision mechanistic exploration
	Rare adverse reaction elucidation	Idiosyncratic mapping	Autoimmune markers		Improves understanding of unpredictable effects
	Long-term stability testing	Chronic, microenvironment	Continuous tracking	biomarker	Enables lifetime exposure simulation
	Full-body human-on-chip simulation	Interconnected kidney, heart, lung	Systemic drug profiling	ADMET	Replaces early-phase animal and clinical testing
	Integration with wearable biosensors	Real-time patient-linked data	Dynamic streaming	biomarker	Personalized remote monitoring
	AI-driven virtual clinical trial models	Digital twin simulations	Virtual patient predictions	cohort	Reduces trial costs and risks

9. Translational and Regulatory Perspectives

The overlap of liver OoC and kidney OoC systems is a dye head in the modern-day toxicology and drug discovery. These microphysiological constructs replicate human-relevant hepatobiliary metabolism, metabolite bioactivation, transporter-mediated disposition, and renal clearance, which are infamously weakly represented in mono-culture 2 -culture cultures or animal models. [136] OoC devices allow real-time, dynamic crosstalk under physiologically relevant flow and shear conditions to enable early, mechanistic discoveries of nephrotoxicity and, hence, preventing expensive late-stage failures in clinical trials as well as allowing faster and more efficient development of safer therapeutics [137-138].

The adoption depends on regulatory approval. The U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA) and OECD agencies are paying increased attention to OoC platforms as the future safety assessment tools. Formal qualification requires extreme reproducibility, mechanistic fidelity, and predictive validity between labs. Significant KPIs are precise separation between nephrotoxic and non-nephrotoxic drugs, loyal recapitulation of human biomarker phenotypes (KIM-1, NGAL, cystatin C) and the loyal analog of nephrotoxic injury pathways, oxidative stress, mitochondrial dysfunction, apoptosis, and fibrotic signaling. Such programs as the FDA Predictive Toxicology Roadmap and NCATS programs are progressively tuning the qualification requirements and performance standards [139-144].

Liver-kidney OoC systems can be used in two ways in drug development pipelines. Early discovery phases take advantage of the high-throughput screening of nephrotoxic liabilities, preclinical studies

are based on metabolite-mediated toxicity and transporter interactions coupled with dose-dependent injury kinetics under controlled shear environments [145]. Simulated data used supplements physiologically based PBPK models, including the gap between in-vitro and clinical results. Besides, iPSCs derived by the patient or primary cells allow unique predictions of nephrotoxicity and tailor-made therapeutic approaches [146]. One of the challenges is standardization; inconsistency, in the form of chip design, cellular sourcing, and microfabrication, may undermine standardization [147]. This is being done by collaborative programs like the IQ Consortium and EUROoCS which provide harmonized SOPs, reference standards and inter-laboratory benchmarking. Ethically, OoC corresponds with the 3Rs, which means reduce, refine, and replace the use of animals, whereas the cost is considered economic since trial wastage and time reduction are reduced [148]. Altogether, liver-kidney OoC technologies represent a mechanistically reactive, ethically acceptable and regulatory-ready approach that is just waiting to fill the gap between preclinical knowledge and clinical application to usher in the next phase of specific toxicology [149].

10. Future Prospects of Liver-Kidney Organ-on-Chip Technology

Liver-kidney organ-on-chip (OoC) introduce the future paradigm of drug discovery, safety testing, and precision medicine that would be the final bridging of the mechanistic-gap between in vitro assays and clinical outcomes. New designs will make possible high-throughput screening (HTS) based on automated microfabrication, parallelized chip arrays, and robotic fluidics and permit simultaneous testing of hundreds of compounds and the creation of dynamic dose-response and toxicity maps. In combination with AI-derived analytics, real-time biomarker tracking, e.g., KIM-1, NGAL, clusterin, will boost the decision-making speed and simplify preclinical pipelines [150]. Individual medicine will be enhanced by patient-derived induced pluripotent stem cells (iPSCs) and primary hepatocytes or kidney tubular cells, which will recap genetic polymorphisms in drug transporter (OATs, OCTs). It allows the prediction of nephrotoxicity risk at the individual level, optimal drug administration, and minimization of adverse events, especially in the area of oncology when the use of nephrotoxic drugs like cisplatin is widespread [151-152].

Integration to form multi-organ, such as cardiac, pulmonary, gut and immune multi-organ platforms including shared microfluidic interfaces, allow physiological relevance, enabling addressing cardiotoxicity, nephrotoxicity as well as systemic ADMET processes. The final goal is a fully incorporated human-on-chip, which is a trusted representative of whole-body pharmacokinetics and pharmacodynamics. Vascularization, scaling and long-term stability remain problematic, however, there is a steady push in the development of microengineering, synthetic biology, and computational modeling to replace animal models, accelerate the transfer of clinical studies, and create therapies with unparalleled specificity [153-154].

12. Conclusion

The biggest challenge in the contemporary drug development is nephrotoxicity, which is responsible to a significant percentage of pre-clinical failures, post-marketing withdrawals, and restriction of therapeutic dosage. It is so complicated because the kidney is of the finest sensitivity to xenobiotics and the liver plays the key role of biotransformation of drugs. Traditional pre-clinical assays such as immortalized cell lines, in vitro monoculture and animal models only capture narrow facets of human renal pathology and poorly predict human renal liabilities until late developmental phases hence the failure of translatability. Mechanistic nephrotoxicity assessment is being revolutionized by the introduction of organ-on-chip technology especially integrative liver-kidney co-cultures. These microphysiological systems mediate important architectural, hemodynamic, and metabolic characteristics of the human organs, and allow physiologically relevant, controllable reproducible interrogation of drug-induced kidney damage. Dual-organ chips are sensitive and specific compared to reductionist models because the presence of real-time oxidative stress, apoptosis and biomarker modulation can be monitored. An example that we give is that of cisplatin and acetaminophen which are clinically relevant agents and the hepatic metabolism is in control of

their renal toxicity. The platform identifies the metabolic sources of the injury displaying the interaction amid reactive metabolites, transporter activities, and signaling cascades.

Translationally, liver-kidney chips have three transformative potentials; (1) improved predictive power, as simultaneous examination of drug metabolism, and renal exposure provides easy access to early identification of high-risk compounds, (2) stronger mechanistic understanding, as non-human testing has been demonstrated to be reduced, and (3) a scalable and ethically acceptable alternative to animal tests that adequately satisfies global requirements to lower the number of high-risk non-human tests but maintain human relevance. Such advantages make the organ-on-chip platforms to be vital assets of modern pre-clinical pipelines. Outside of toxicity screening, patient-derived cells can be added to these systems to provide individual risk stratification and integrations with such high-content imaging, omics, and artificial intelligence will provide unprecedented datasets to remake drug safety paradigms. Organ-on-chip data is increasingly accepted by regulatory bodies, and after standardization and validation, there is a high probability that organ-on-chip data would expedite decision-making operations. Overall, integrative liver-kidney chips will fill the gap between reductionism and human pathophysiology and mark a new dawn of specific nephrotoxicity predictive capacity and safer drug development.

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