Organ-on-chips: increased complexity for higher physiological relevance in pharmaceutical safety testing.

With as many as 90% of new drug compounds falling short in clinical trials, the journey from a promising drug candidate to a marketable medication is fraught with challenges and uncertainties. This high attrition rate is not only costly for drug developers but also hinders access to better and potentially life-saving treatments for patients. Even with innovative tools such as artificial intelligence, machine learning, 3D cell cultures, and omics-based technologies, the prevalence of drug failures continues to cast a shadow on the promise of breakthrough therapies.

One of the major reasons for the failure of many new medicines is the inability to accurately predict drug responses *in vivo*. Although animal models are widely used for preclinical and toxicity testing, the results obtained often inaccurately reflect human physiology due to interspecies differences. Animal studies are also costly and time-consuming and have raised ethical and animal welfare concerns. Despite considerable advances in understanding the pathophysiological differences and similarities between patients and preclinical animal models, there are still instances of over- or underestimating cellular behaviors and drug responses in preclinical phases, especially when addressing efficacy and toxicity. This discrepancy has resulted in drugs that successfully pass through preclinical development only to fail in later clinical stages.

A notable step in addressing the reliance on animal testing was the U.S. Congress's passage of the FDA Modernization Act 2.0 in 2023. This authorizes the use of certain alternatives to animal testing for drug and biological product applications, such as cell-based assays and computer models, to investigate the safety and effectiveness of a drug. The legislation also removes a requirement to use animal studies as part of the process to obtain a license for a biological product that is biosimilar or interchangeable with another biological product.

While these efforts are a step forward from an ethical standpoint, it also presumes that alternative research models are available for preclinical testing. One easily accessible alternative involves *in vitro* 2D cell culture models using primary or immortalized cell lines. The challenge with these models is that they cannot faithfully replicate the physiological conditions of *in vivo* cellular environments, such as the spatial organization, dimensionality of the extracellular matrix (ECM), and critical cell-cell and cell-ECM interactions. There is therefore a need for the development of accurate and reliable *in vitro* models with acceptable biological relevance to accelerate the development of new drugs, while also mitigating the risk of costly failures. These models are required to bridge the gap between traditional testing methods and *in vivo* biology, ensuring a seamless transition from promising candidates to efficacious medications for patients.

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Modeling toxicity with organ-on-chip platforms

Organ-on-chip (OOC) models have emerged as a promising alternative, potentially overcoming some of the limitations of animal experiments. An OOC is a microfluidic device containing hollow channels lined with living cells, which are often derived from human tissues or stem cells, to form miniature organ-like structures. The cells can interact with each other and the microenvironment to mimic functional units of specific organs. The primary goal of OOC technology is to replicate the complex microenvironment of human organs *in vitro,* allowing researchers to study diverse physiological processes, test drugs, and gain insights into human biology. One of the attractive features of OOC devices is their ability to reproduce mechanical forces and physical characteristics unique to each organ. For example, lung-on-chip devices may simulate breathing motions, while heart-on-chip devices may replicate the heart's rhythmic contractions.

Because the failure of many drug candidates is often attributed to their toxicity in humans, with the liver and kidneys being common sites of adverse effects, leveraging OOCs in drug safety assessments allows researchers to closely mimic the physiological conditions of these vital organs *in vitro.* This approach can provide valuable insights into potential toxicities and help to identify compounds with improved safety profiles early in the drug development process. In this article, we explore some advancements of liver and kidney-on-chip models, illustrating how these platforms empower researchers to simulate toxicity responses.

Liver-on-chip models for evaluating hepatotoxicity

Drug-induced liver injury (DILI) is one of the primary toxicities that cause drugs to be withdrawn from the market and a major safety concern in pharmaceutical development. Increasing numbers of liver-on-chip platforms are being developed to model drug metabolism, drug-drug interactions, and hepatotoxicity. These devices typically contain human liver cells, such as hepatocytes, and are designed to mimic the structural and functional complexity of the liver tissue. While liver-on-chip technology holds promise for advancing drug development, the models need to replicate the liver's histological structures and functions and accurately distinguish between toxic and non-toxic drugs.

In a study conducted by Ewart *et al.,* the team investigated the efficacy of a liver-on-chip platform (Liver-Chip) in recapitulating the human liver structure and predicting DILI.[1] Successful mimicry of the structural and cellular organization of the human liver increases the likelihood that the device will also replicate its functional capabilities.

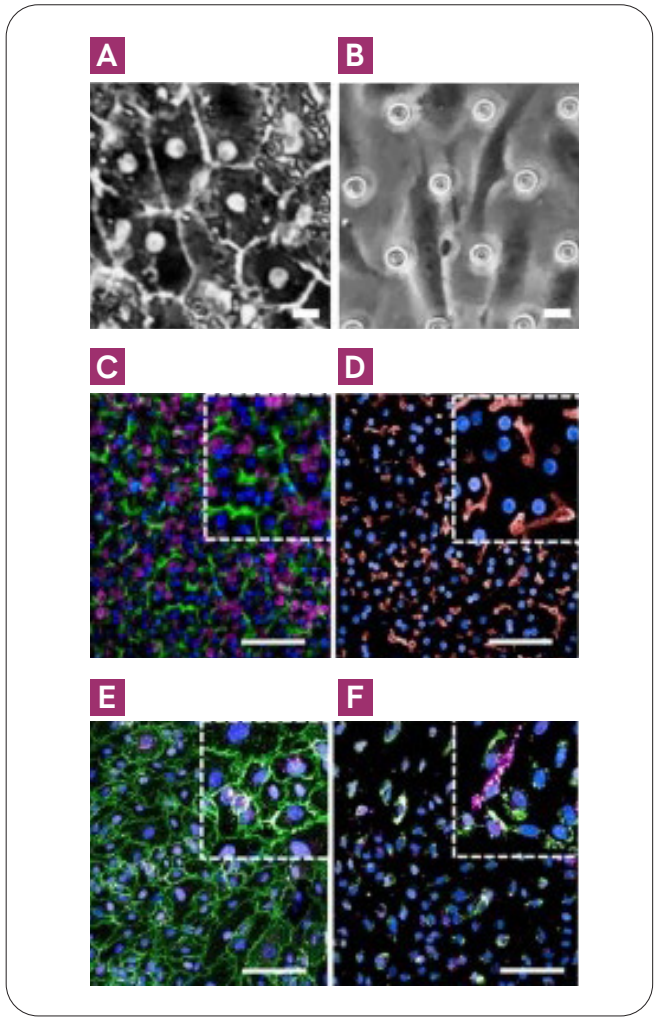

Figure 1: Recapitulation of human liver structure in the Liver-Chip. Representative phase contrast microscopic images (scale bar represents 10 µm) of hepatocytes in the upper channel of Liver-Chip (a) and non-parenchymal cells in the lower vascular channel (the regular array of circles are the pores in the membrane) (b). Representative immunofluorescence microscopic images showing the phalloidin stained actin cytoskeleton (green) and ATPB-containing mitochondria (magenta) (c) and MRP2-containing bile canaliculi (red) (d). CD31-stained liver sinusoidal endothelial cells (green) and desmin CD68+ containing stellate cells (magenta) (e), and Kupffer cells (green) co-localized with desmin-containing stellate cells (magenta) (f). All images in c–f show DAPI-stained nuclei (blue) and the scale bar represents 100 µm with the inset at 5 times higher magnification. Image source: Ewart L, Apostolou A, Briggs SA, Carman CV, Chaff JT, Heng AR, et al. Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology. *Communications Medicine*. 2022;2(1). Image licensed under Creative Commons License 4.0.

Leveraging the Opera Phenix® high-content screening system the researchers confirmed the presence of distinct hepatocytes and liver sinusoidal endothelial cells (LSECs) within the Liver-Chip (Figures 1a-b). They also observed liver-specific structures such as bile canaliculi, mitochondrial markers in hepatocytes, and specific cellular markers for LSECs, Kupffer cells, and stellate cells (Figures 1c-f). Once they had confirmed the model's ability to sustain hepatocyte functionality, the team investigated the ability of the liver-on-chip platform to predict DILI across a blinded set of 27 drugs, each with a known hepatotoxic or non-toxic behavior. The Liver-Chip correctly identified 87% of drugs causing DILI, outperforming both 3D liver and animal models. Additionally, no drugs were falsely marked as toxic, highlighting the potential of liver-on-chip models to identify hepatotoxic compounds and revolutionize drug development processes. Beyond the scientific validation, the researchers also noted the profound economic implications of adopting such technologies due to increased R&D productivity. This suggests that liver-on-chip models could improve the efficiency of drug development and also offer substantial economic benefits.

Kidney-on-chip models for evaluating nephrotoxicity

In addition to DILI, drug-induced nephrotoxicity remains a significant concern in pharmaceutical development and has led to the withdrawal of various medications from the market due to unforeseen renal complications. Early detection of potential nephrotoxic effects is therefore critical not only to avert costly late-stage setbacks but also to aid the development of safer therapeutic options for patients.

Central to effective nephrotoxicity testing is the ability to develop physiologically relevant models of the proximal tubule (PT). This site of the nephron is crucial for drug clearance and is one of the primary sites susceptible to drug-induced renal damage. Although various animal and *in vitro* models of the kidney exist, they often fail to capture the intricate functions and responses of the PT to drug exposures. The emergence of advanced kidney-on-chip platforms therefore offers a promising avenue for more physiologically relevant nephrotoxicity testing. Yet, replicating the PT's structure and function poses specific challenges, especially given that reabsorption within the PT occurs across opposing monolayers of epithelium and endothelium separated by a basement membrane.

A study conducted by Vedula and colleagues involved the development of a kidney-on-chip model designed to address these complexities.[2] The model integrated human renal PT epithelial cells (hRPTEC) and human microvascular endothelial cells (hMVEC) within a co-culture setting. The setup aimed to facilitate the formation of PT tissue structures that closely resemble the *in vivo* environment while also allowing for real-time quantification of renal reabsorptive functions. After confirming that the co-culture architecture successfully mimicked the physiological structure of a renal PT, the researchers used a fluorescent glucose analog (2-NBDG) to monitor reabsorption in real-time in the presence of the Na^+/K^+-ATPase inhibitor, ouabain. The dynamic responses in glucose reabsorption the team observed validated the model's ability to reproduce a crucial function of the PT. The researchers note that, unlike previous models, their microfluidic PT model not only mimicked the physiological architecture but also provided direct evidence of active reabsorptive functionalities across epithelial and endothelial cell monolayers.

Building on this work, another research effort led by Erin Shaughnessey evaluated the use of a kidney-on-chip model to detect drug-induced nephrotoxicity in primary PT cells, leveraging the high-throughput PREDICT96 microfluidic system.[3] The PREDICT96 platform enables parallel culture of 96 individual co-culture devices and control of fluid flow within the footprint of a conventional multi-well culture plate (Figure 2). Through this platform, the researchers were able to evaluate the effectiveness of transepithelial electrical resistance (TEER) sensing in identifying cisplatin-induced toxicity within human primary PT models. Their investigation spanned various conditions, including mono- and co-culture settings and differing levels of fluid shear stress (FSS).

While both models responded to cisplatin-induced toxicity, only the hRPTEC-hMVEC co-culture model showed TEER changes correlating with cytotoxicity and tight junction alterations. Importantly, the TEER measurements within the co-culture model indicated the emergence of cisplatin-induced toxicity earlier than observable cell death, showcasing its potential as an early, non-invasive indicator of drug-induced nephrotoxicity in a high-throughput screening setting. Such findings position the PREDICT96 platform as a pivotal tool in high-throughput nephrotoxicity screening endeavors and underscore the potential of TEER as an early and non-invasive biomarker for detecting drug-induced nephrotoxicity.

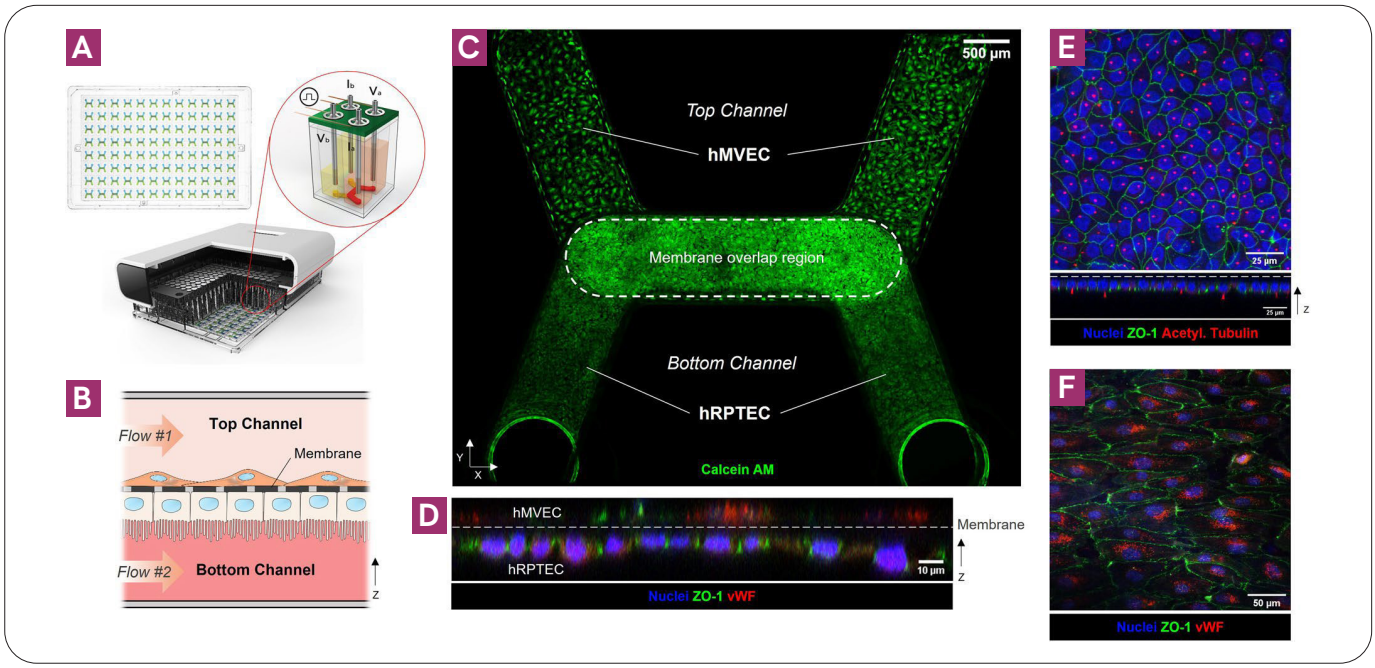

Figure 2: The PREDICT96 platform with integrated TEER sensing supports proximal tubule-microvascular co-culture ***in vivo-like*** features. (a) The PREDICT96 culture plate has 96 microfluidic devices (top left). Cross-sectional rendering of PREDICT96 Integrated TEER system (bottom) highlights the four-point TEER measurement unit (illustration, top right) in which the stainless steel pump tubes double as electrodes. (b) A cross-section schematic of the co-culture kidney model in the bilayer microfluidic device with hRPTEC on the bottom of the membrane and hMVEC on top of the membrane. Fluid flow is controlled separately in the top and bottom channels. (c) A confocal tile scan of a PREDICT96 device shows confluent cell layers under high FSS (0.70 dyn/cm^2) on day 7 (Calcein AM, green). (d) An orthogonal view of a confocal z-stack shows hRPTEC and hMVEC on either side of the device membrane (dashed line) with hRPTEC expressing apical ZO-1 (green) and hMVEC expressing endothelial marker von Willebrand factor (vWF, red). (e, top) A maximum intensity projection of a z-stack of hRPTEC on the bottom side of the membrane shows continuous tight junctions (ZO- 1, green) and abundant primary cilia (acetylated tubulin, red). (e, bottom) An orthogonal view demonstrates hRPTEC apical expression of ZO-1 and primary cilia. (f) A confocal z-slice of hMVEC on the top side of the membrane shows a characteristic punctate expression of vWF (red) and tight junctions (green). Image source: Shaughnessey EM, Kann SH, Azizgolshani H, Black LD, Charest JL, Vedula EM. Evaluation of Rapid Transepithelial Electrical Resistance (teer) measurement as a metric of kidney toxicity in a high-throughput microfluidic culture system. *Scientific Reports*. 2022;12(1). Image licensed under Creative Commons License 4.0.

Future outlook

Advancements in OOC models have enabled microfluidic platforms to emulate the microenvironments of various human organs with remarkable fidelity. Particularly, liver- and kidney-on-chip platforms are enhancing the predictive accuracy of drug-induced toxicities such as hepatotoxicity and nephrotoxicity, respectively. The microfluidic systems in OOC devices enable the continuous perfusion of nutrients, oxygen, and other essential factors to the cells. This perfusion system helps maintain cell viability and function over extended time periods, potentially allowing for studies involving longer-term processes, such as chronic drug exposure, or disease progression. By utilizing high-content imaging, OOCs can provide detailed visualizations of cell interactions, structures, and responses within the microfluidic devices, enhancing the accuracy of drug toxicity assessments and facilitating the development of safer and more effective medicines.

As the scientific community becomes more aware of the advantages of OOCs over traditional models, their adoption will likely continue to increase. This will be driven by the pressing need to reduce drug development costs, improve success rates, and address ethical concerns related to animal testing. With growing interest from pharmaceutical companies, we can expect more significant investments in OOC technologies leading to the development of standardized OOC platforms, making them more accessible and widely adopted across the industry.

References

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