Recreating Life in the Lab: How Predictive Human Organ Models are Transforming the Efficiency of Drug Discovery

An often-underreported fact within the drug discovery and development industry is that around 90% of drug candidates reaching clinical trials ultimately fail, with an even greater number discarded before reaching the clinic at all. A recent market survey of mid-sized biotechs and large pharma companies highlighted efficacy and cost as the leading concerns during drug development, further demonstrating the impact that failed drugs can create. These failures are predominantly caused by a lack of efficacy, but unforeseen adverse effects in patients are also a factor for concern. Clearly, there is an urgent need for more translatable data between the preclinical and clinical phases of drug discovery and development to address the financial uncertainty caused by the high degree of drug candidate failure.

The NAM Revolution

Scientists now have access to a large range of preclinical tools for evaluating drug safety and efficacy, including simple *in vitro* 2D/3D cell culture assays and *in vivo* animal models. The former is convenient and scalable, enabling large numbers of candidates to be screened rapidly but these assays lack physiological relevance. The latter compensates by providing the complexity of a living system; however, animal models lack human relevance. A new wave of technologies, collectively called New Alternative Methods, or New Approach Methodologies (NAMs), aim to bridge this gap by modeling the physiological processes that occur in our organs and systems. NAMs are defined as any technology, methodology, approach, or combination that can provide information on drug hazard and risk assessment and avoid the use of animals. They include *in silico*, *in chemico*, *in vitro*, and *ex vivo* approaches. The complementary role of NAMs in drug discovery and development has become increasingly apparent following the FDA's Modernization Act 2.0. The "Alternatives to Animal Testing" bill now allows the FDA to consider data generated from non-animal drug testing methods in IND submissions, where enhanced performance is proven.

An Introduction to Organ-on-a-chip

Organ-on-a-chip (OOC) technology has gained rapid traction within the NAM market over the past decade. OOCs, also referred to as microphysiological systems (MPS), were first described in 2010 with Harvard University's lung-on-a-chip model, derived from microfluidic devices that assisted academics with cell culture.[1] This has since paved the way for the commercial development of many additional organ models and technology providers.

OOC technologies generate 3D microtissues that recapitulate the microarchitecture, functions and physiological responses of human organs and tissues more accurately than conventional preclinical models. 3D microtissues are grown by co-culturing organ-specific primary human cells in the presence of microfluidic perfusion (to mimic the bloodstream), providing biomechanical stimuli, oxygen, nutrients and waste removal. Furthermore, OOC technology enables complex stimuli such as growth factors to activate cellular processes, interferons to trigger an immune response, fat loading to mimic western diets, and drugs to predict their human effects.

The capacity to recreate an environment that more accurately represents the human body makes OOC useful in almost every step of drug discovery and development. When combined with existing methodologies (and other NAMs), OOC provides human relevant insights that can supplement, cross-validate, or query existing datasets, providing a "bigger picture" for more informed decision making. For this reason, independent research suggests that 26 % of all R&D costs will be saved where OOCs are integrated into Pharma workflows.[2] Market research supports this hypothesis, highlighting the top three reasons for OOC purchase; to reduce costs, improve the human translatability of results, and detect/recover flawed drugs.

OOC Applications Across the Preclinical Landscape

In early discovery, OOC technology facilitates a deeper understanding of human physiology and disease mechanisms to support target identification/validation. Here, OOCs complement patient-derived clinical samples, animal models and other *in vitro* preclinical tools by corroborating target-specific data, or unlocking new avenues to explore. The insights this provides are highly sought after by drug developers.

The same OOC models can also be used within lead optimisation to complement and inform *in vivo* efficacy studies by enabling a larger range of conditions to be explored ahead of costly animal studies. By allowing the effective therapeutic dose range to be refined ahead of time, the approach supports a reduction in the number of animals required.

During this drug discovery phase, OOCs are also utilised to generate toxicology profiles. Drug-induced liver injury (DILI) remains a major contributor to late-stage drug failures and market withdrawal, however, it is possible to de-risk the process by incorporating liver-on-a-chip models into the preclinical toolbox. Offering enhanced performance versus standard techniques,[3] liver-on-a-chip provides a more sensitive way to uncover potential adverse effects early enough to recover promising, but flawed, drugs.

Further along the development pipeline, these predictive human models can be used alongside animal studies to confirm, or query, unexpected drug efficacy, toxicity, or Absorption, Distribution, Metabolism and Excretion (ADME) results. Here, OOC models reduce risk of false reporting due to interspecies issues. In certain cases, OOCs provide a direct alternative to animals – especially where translatability to humans is poor. Human-specific drug modalities (e.g., cell, antibody and gene therapies), for example, pose a significant development challenge. Interspecies differences in genetics, metabolism, or

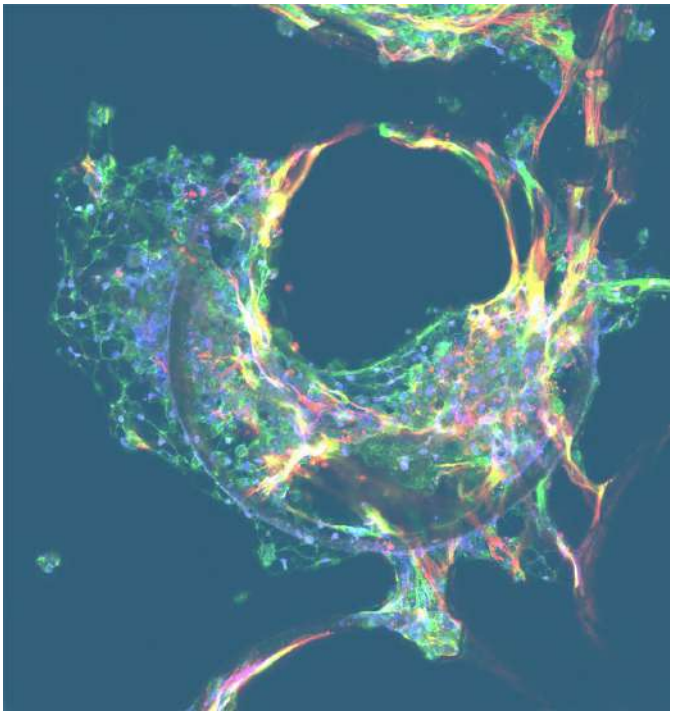
immunological response render animal models less suitable for safety and efficacy testing when studying advanced therapies. Similarly, when predicting immune-mediated, or idiosyncratic toxicity, OOC offers significant benefits when compared to conventional methods. By incorporating tissue-specific immune cells and peripheral blood mononuclear cells (PBMCs), OOC models recapitulate elements of the human adaptive and innate immune response to provide improved assay sensitivity. Should adverse effects pass undetected within discovery and be reported during clinical trials, the final use of OOCs can be to recreate the clinical scenario and help unlock the cause.

OOC in the Lab

Adopting OOC models into drug discovery and development workflows is a straightforward process, with a variety of hardware, consumables, kits, and pre-validated cells commercially available. Furthermore, OOC models are a highly versatile option, enabling users to precisely adjust the type and ratio of primary human cells to recreate a broad spectrum of environments. For example, simple primary human hepatocyte (PHH) monocultures are used in isolation to overcome the limitations of standard drug metabolism assays to predict the human *in vivo* clearance rates of slowly metabolised drugs, or to identify rare or human-specific metabolites.[4]

To model more complex systems that can recapitulate human immune responses, duo-cultures of PHH and Kupffer cells allow researchers to investigate immune-mediated toxicity in DILI assays5. The model can be further enhanced with the addition of circulating PBMCs into the flow that perfuses organs to flag idiosyncratic toxicity.[6]

To recreate common, but therapeutically un-met, metabolic liver diseases such as Non-alcoholic steatohepatitis (NASH), a co-culture of PHH, Kupffer and human stellate cells are grown to form a liver microtissue that is subsequently exposed to fat loading, thus inducing disease state. In 2021, Kostrzewski *et al.*,[7] demonstrated that the model accurately recapitulates



key aspects of the human condition, whilst Vacca *et al.*[8] used transcriptomic profiling to demonstrate that the model more closely replicates changes found in NASH patients than the conventional murine WD model. This same predictive human model can be used to identify increased DILI susceptibilities for patients with the underlying disease to reduce the risk that therapeutics exacerbate the pre-existing condition.

Liver-on-a-chip tissues are metabolically active, functional and ready for experiments after four days of culture. Each liver chip can be studied for up to four weeks, depending on the needs of the application, and the dosing schedule. For toxicity studies, optimal results can be obtained over eight to ten days, however, disease modeling and efficacy studies may benefit from extended periods to observe longer-term drug effects. As such, these models provide a faster, more human-relevant, and cost-effective approach versus animal models.

In addition to these benefits, many OOC models enable imaging and/or sample recovery to be performed periodically throughout experiments, delivering high content longitudinal data to measure responses such as metabolite formation, biomarker production, or phenotypic responses. Data from OOC assays are generated using standard laboratory techniques such as microscopy, histology cytometry, sequencing, mass spectrometry and multiplexed immunoassays to produce rich insights from each sample. Studies from high profile groups such as the FDA have highlighted the robustness of OOC data; confirming the reproducibility of drug response data collected from two distinct batches of primary human Kupffer cells at multiple test sites using the PhysioMimix® liver MPS and the superior performance of this approach for drug metabolism, toxicity and accumulation applications relative to *in vivo* animal models.[3]

Beyond culturing single-organ models in isolation, it is now possible to interconnect OOC models together into fluidically linked multi-organ systems. Integrating liver models with other models such as the lung or gut, for example, recapitulates common routes of drug administration. Multi-organ systems simulate processes such as drug absorption and metabolism to predict bioavailability and provide preclinical, human-relevant insights. In conjunction with physiologically based pharmacokinetic modeling OOC data can be extrapolated from an *in vitro* result into an *in vivo* prediction to inform dose setting. Multi-organ models also provide a means to facilitate interorgan crosstalk for evaluating on- and off-target drug effects or to interrogate the effects of inflammation or other risk factors when investigating drug effects.

A Look to the Future

As such, OOCs represent a concrete approach to reducing, refining, and complementing existing drug testing methodologies. With market research showing that 66% of respondents are currently using, or plan to adopt OOC in the next two years, these technologies are set to transform the way that drugs are discovered and tested by enabling *in vitro* recapitulation of human physiology. They provide lower cost, higher throughput animal alternatives that can replace or complement, as needed. By improving the translatability of data between the laboratory and the clinic, more accurate predictions of human responses can now be made in the

discovery and development phases to decrease the risk of unexpected failures during human clinical trials. Ultimately, the field still strives towards the end goal of the 'body-on-a-chip', where multi-organ systems can accurately replicate precise genetic or gender differences to support development of advanced, personalised therapies. Only time will tell if the approach saves incalculable time and billions of dollars in research and development costs alongside additional NAMs, such as *in silico* modeling and AI, but right now the future for OOC looks bright.

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