

# Novel Models for Drug Discovery



  
charles river

# Novel In Vitro Models for Drug Discovery Technologies and Trends

By Ryan Feaver, D. Lansing Taylor and James B. Hoying

The difficulty of replicating complex human biology in preclinical studies can make drug development a challenging process. Even compounds that appear effective when tested in cell cultures and animal models often fail in humans—an estimated 90 percent of drug candidates entering Phase I clinical trials never reach approval.<sup>1</sup>

To improve this situation, efforts are underway to create new *in vitro* models that accurately replicate human biology. These human microphysiology systems (MPS) utilize human cells<sup>2</sup> to create tissues, taking advantage of the advances in using induced pluripotent stem cells (iPSCs) to create specific cell types from patient-derived skin and/or blood cells.

These solutions have found extensive application in modeling liver diseases, including the liver as a niche for metastatic cancers and non-alcoholic fatty liver disease (NAFLD), an emerging health crisis that affects up to one in three Americans, three of four patients with type 2 diabetes and 95 percent of obese individuals. NAFLD can progress to non-alcoholic steatohepatitis (NASH), cirrhosis and ultimately liver cancer, often resulting in liver transplant or death.

## NASH in a Dish

Despite being one of the most active areas in drug development, with over 40 candidates in the preclinical and clinical phase, no candidates to treat these liver diseases have yet advanced beyond Phase III clinical trials, much less received regulatory approval. That is in large part because the biological mechanisms driving the progressive stages of NAFLD and NASH are poorly understood; current *in vitro* or *in vivo* models poorly mimic the pathophysiology and biological hallmarks of NASH.

One of us describes an *in vitro* approach to create “NASH in a dish” by combining primary human hepatocytes, stellate

cells, macrophages in a physiologically relevant tissue microenvironment consisting of liver sinusoid hemodynamic and transport conditions, supplemented by clinically-derived concentrations of NASH risk factors.

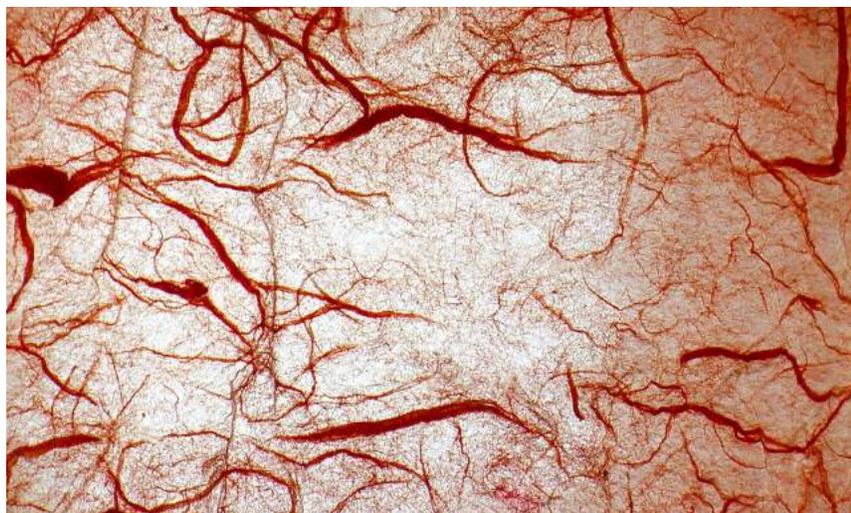
This system captures critical pathophysiological drivers of NASH such as steatosis, inflammation, and fibrosis and has been validated against human NASH biopsy samples and with leading clinical stage drugs.<sup>3</sup> The system is able to replicate clinical lipidomic responses measured from biopsies in NAFLD and NASH patients. This *in vitro* human NASH model has been used to survey the current drug development landscape in order to identify new targets for NASH therapies.

This “patient in a dish” concept is also being applied to identify novel targets and develop new therapies to treat

pediatric rare liver diseases<sup>4</sup> and, in the future, rare vascular diseases.<sup>5</sup>

## Quantitative Systems Pharmacology

One of us has also embraced a Quantitative Systems Pharmacology (QSP) approach that integrates and iterates experimental and computational methods in drug discovery and development programs, especially in metastatic breast cancer, neurodegenerative diseases and liver diseases.<sup>5</sup> The experimental part of QSP is being advanced through the use of MPS containing iPSC-derived cells from patients, which incorporates the biologically relevant heterogeneity that is a major challenge in discovery.<sup>6</sup> It is expected that the MPS will become a standard component in the QSP-driven approach to drug discovery and development.





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A human, 3-D, four-liver-cell type, microfluidic MPS has been developed to investigate absorption, distribution, metabolism, excretion and toxicology (ADME-TOX) as well as human liver diseases. It includes fluorescence-based biosensors for real-time imaging of spatial and temporal changes in the production of reactive oxygen species (ROS-), induction of apoptosis and the migration of cells within the MPS<sup>7,8</sup> for investigations lasting a month or more. Individual organ MPS are also being coupled to investigate the multi-organ impact on ADME-TOX and disease progression in intestine, liver, kidney, muscle and the blood-brain barrier.<sup>9</sup> Critically, a microphysiology database has been developed for the acquisition, management, analysis, validation and modeling of data.<sup>10</sup>

Human liver MPS models have the potential to allow the progression of metastasis to be explored and to test therapeutic treatments.<sup>11</sup> The liver is a common site of cancer metastasis, often with a very poor outcome, and the complex steps involved in extravasation and cell seeding to initiate liver involvement are complex, including the process of dormancy. Furthermore, animal models of metastasis have not been concordant with human disease.

## Modeling Microvasculature

A comprehensive picture of human disease also requires consideration of microvascularization, which plays a role in conditions such as heart attack, cancer and diabetes. With this in mind, one of us has developed an *in vitro* microvasculature model intended to capture as many aspects of microvascular complexity and the native angiogenesis process as possible, including all of the relevant cell types and vessel structure—without sacrificing experimental simplicity and cost effectiveness.

This model system has been used to identify and characterize angiogenic factors and inhibitors, evaluate micro-

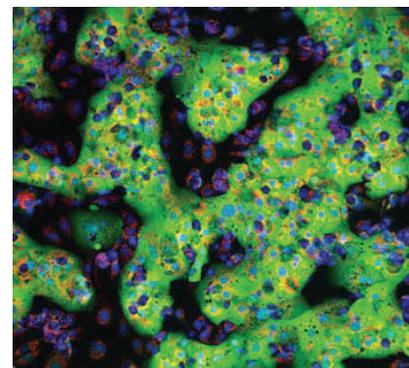
vascular instability and characterize tissue dynamics during angiogenesis. By using the full microvasculature, it captures biological processes in a way that cell cultures cannot. As an assay, the system can help identify drug candidates by screening out those compounds that are toxic or not effective and eliminating false positives from consideration.

This assay was used to perform a phenotypic screen of 128 compounds targeting a variety of epigenetic regulators for their effects on angiogenesis. The screen used 3-D cultures of isolated microvessels under serum-free conditions that modestly promote angiogenesis, enabling us to identify agents that either stimulate or inhibit angiogenesis.<sup>12</sup>

These techniques are already being demonstrated as effective alternatives to animal and cell culture models that can greatly enhance the drug discovery process. Ultimately, these tools may help realize some of the goals of precision medicine, by enabling us to comprehensively model an individual patient's biology for the purposes of testing effectiveness and identifying adverse reactions.

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# Non-Traditional Animal Models for Drug Discovery

By Kevin Strange  
and Shaochen Chen

Understanding disease and discovering drugs to treat it often requires innovative approaches.

**W**e describe several examples of novel models and tools that are being deployed in drug development: zebrafish used to discover a small molecule useful in regenerative medicine, a 3-D bioprinting technique that can create vasculature needed for tissue and organ survival and probiotic bacteria that can be genetically programmed to treat disease.

## Zebrafish and Regenerative Medicine

When it comes to regenerative medicine, one of us proposes that “non-traditional” animal models such as zebrafish present the most economical and efficient strategy for development of small molecules in both target- and phenotype-based drug discovery.

Humans and other mammals have limited capacity for regenerating damaged tissues, even though they possess the necessary genetic instructions. In contrast, many invertebrates and lower vertebrates exhibit remarkable regenerative capabilities. The zebrafish, which can fully regenerate many lost

or damaged body parts, was used for phenotype-based small molecule screening and discovery.

Researchers trying to develop small molecules capable of activating endogenous tissue regenerative mechanisms used the zebrafish as a drug screening platform. Molecules that stimulated tissue regeneration in zebrafish were subsequently shown to be effective in mice. One of these molecules, MSI-1436, is the first and to date only small molecule capable of inducing regeneration in the adult mammalian heart following ischemic injury.<sup>1</sup>

## 3-D Bioprinting Blood Vessels for Tissues and Organs

One of the biggest challenges in tissue engineering is to create lifelike tissues and organs with functioning vasculature that can integrate with the human body. The problem is due largely to the difficulty in creating blood vessels that allow tissues and organs to survive and work properly.<sup>2</sup>

Working in mice, researchers have used rapid continuous projection 3-D

bioprinting to create scaffolds incorporating a variety of biomaterials to control micro-architecture, mechanical, chemical and biological properties.<sup>3</sup> A prevascularization technique uses this rapid 3-D bioprinting method to create multiple cell types mimicking the native vascular cell composition, which are precisely encapsulated directly into hydrogels.<sup>4</sup> *In vivo* implantation has demonstrated the survival and progressive formation of the endothelial network in the prevascularized tissue. Anastomosis between the bioprinted endothelial network and host circulation has been observed, with functional blood vessels featuring red blood cells.

Such functional biomaterials allow researchers to investigate cell-micro-environment interactions at nano- and microscales in response to integrated physical and chemical stimuli. This allows the creation of both *in vitro* and *in vivo* microphysiological systems, such as human liver tissue, for tissue regeneration, disease modeling and drug discovery.<sup>5</sup>

With its superior bioprinting speed, flexibility and scalability, this new prevascularization approach can be broadly applicable to the engineering and translation of various functional tissues. Work is underway to build patient-specific tissues using human induced pluripotent stem cells, which would protect transplants from attack by a patient's immune system.



### Genetically Engineering Bacteria to Fight Disease

Another innovative approach relies on genetically engineering probiotic bacteria to perform specific therapeutic functions with established links to disease. These synthetic biotic medicines, taken orally, act in the gut microbiome to correct missing or dysfunctional metabolic activities throughout the body. Synthetic biotic medicines can be delivered to the site of disease in cases where local activity is critical, as in stimulation of immune effectors to combat certain tumors.

Work is being done to treat patients with rare genetic metabolic diseases, including urea cycle disorders (UCD) and phenylketonuria (PKU), as well as to address more prevalent conditions

such as inflammatory bowel disease (IBD), cancer and metabolic conditions.

These examples illustrate the value of thinking outside the box in the search for new drugs. By breaking with tradition, these approaches are expanding both the researcher's toolbox and the boundaries of our clinical capacity to treat disease.

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