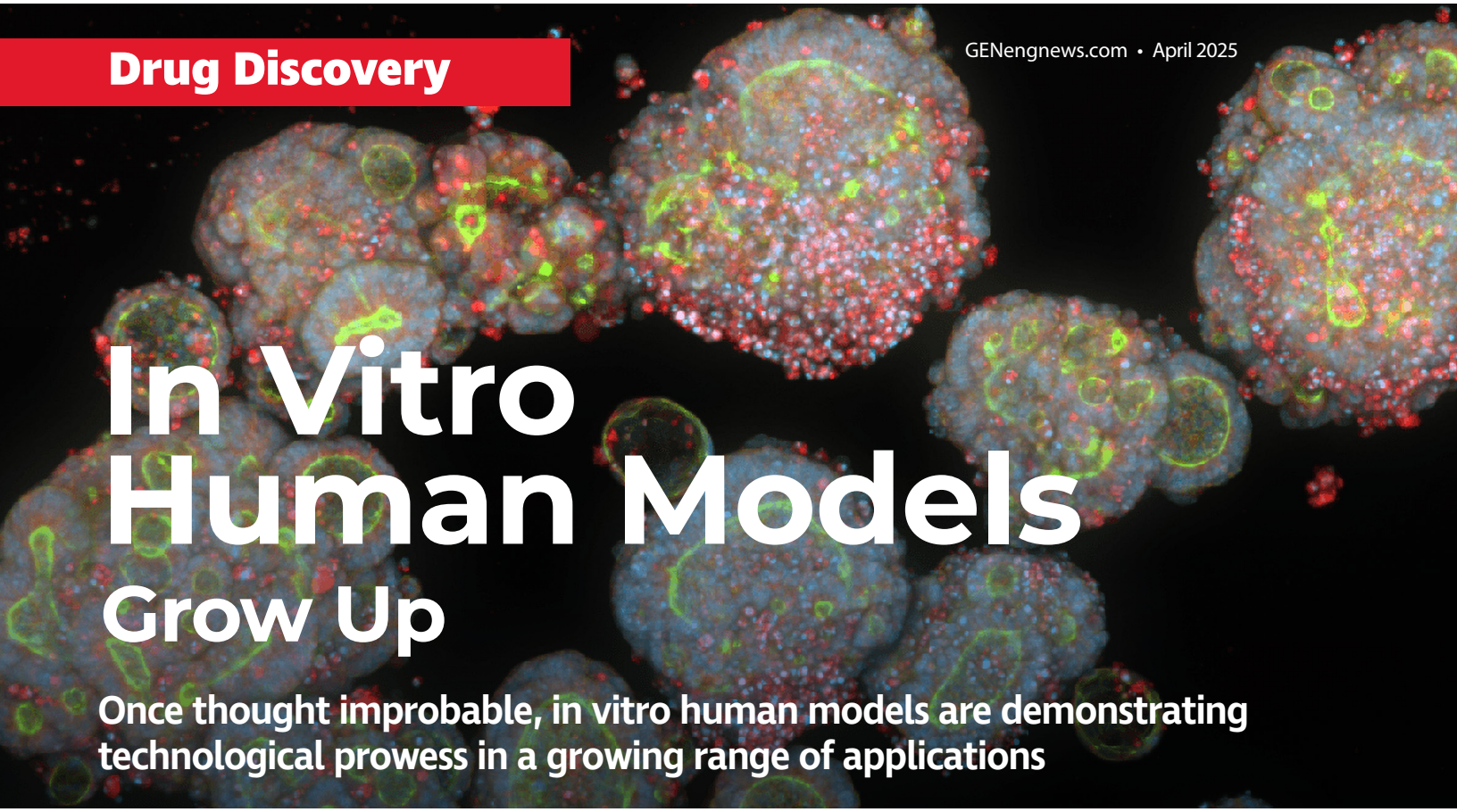
A REPRINT FROM

GEN Genetic Engineering & Biotechnology News

Drug Discovery

GENengnews.com • April 2025



In Vitro Human Models Grow Up

Once thought improbable, in vitro human models are demonstrating technological prowess in a growing range of applications

By MaryAnn Labant

For decades, animal models have been the gold standard for preclinical testing of potential drugs. Yet, even the best animal models are not perfect. For example, small rodent species with engrafted human immune systems and edited genes will still never be completely human. To address this shortcoming, 3D cell culture enthusiasts are tackling the challenge by developing robust in vitro human models.

Organoids, microphysiological systems (MPS), and organ-on-a-chip (OOC) devices continue to make steady progress and proceed down the path to technology maturity. Innovative developers are refining and validating their offerings to fit particular testing questions, such as drug-induced liver injury (DILI).

Although these in vitro models will also not substitute totally for their in vivo counterparts, the benefits over non-human models are clear. The FDA Modernization Act 2.0 and the proposed FDA Modernization Act 3.0 support the use of alternative methodologies in regulatory submissions. Current limitations of in vitro human models do not take away from their ethical reduction of animal use nor their potential to provide more realistic in vitro human responses in preclinical testing.

As in any emerging technology, more work remains to be done, and developers are responding positively. And virtually everyone agrees that standardization of nomenclature and characterization to ensure consistent and reproducible results can only facilitate the greater acceptance of these models into regulatory guidance.

Open platforms

"Our microfluidic system is an open platform that enables users to incorporate virtually any type of cell within our portfolio of Organ-Chip consumables," said Lorna Ewart, PhD, CSO at **Emulate**. "Each consumable type has the same footprint. This compatibility gives the platform an incredible amount of flexibility in the types of biology that can be modeled."

The initial Emulate Organ-Chip design—the Chip-S1® Stretchable Chip—

Above. Patient-derived colorectal cancer organoid stained with Hoechst (*blue*) for nuclei, phalloidin (*green*) for actin filament, and MitoTracker (*red*) for mitochondria. Images were acquired using the ImageXpress HCS.ai High-Content Screening System equipped with a confocal spinning disk. Molecular Devices

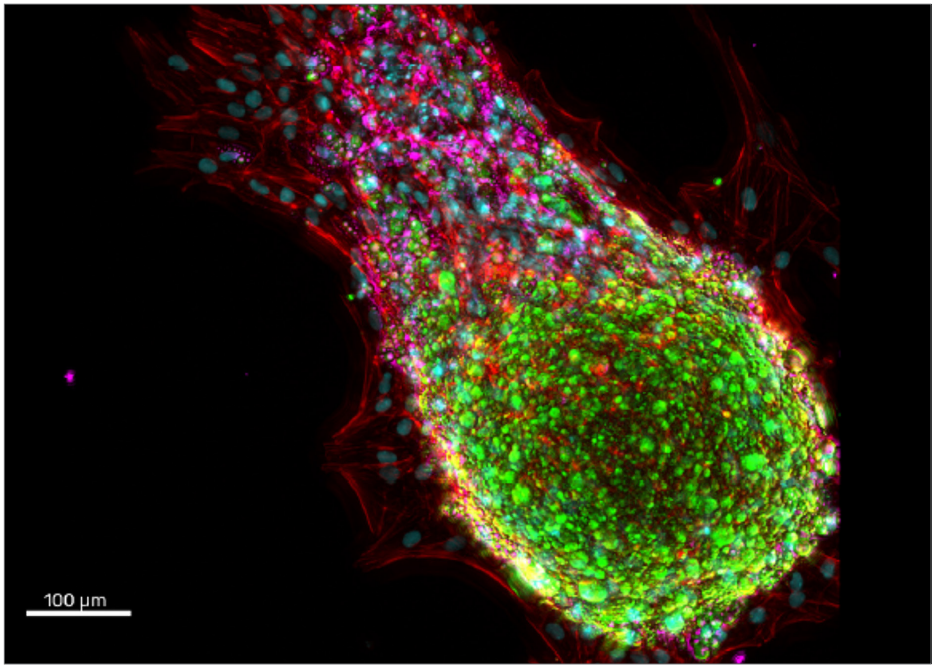
is composed of PDMS, a transparent and gas-permeable viscoelastic polymer material that is well suited for modeling the in vivo microenvironment. Notably, Emulate was the winner of the 2024 Science Lush prize for work on their human Liver-Chip, built on the Chip-S1, for predicting DILI in preclinical toxicology.

But in some instances, PDMS can absorb lipophilic drugs, potentially leading to inaccurate compound dosing and thus misrepresentation of safety or efficacy. This prompted the development of the Chip-R1™ Rigid Chip, which is made from minimally drug-absorbent plastic, especially amenable for ADME, toxicology, and efficacy studies. The smaller height of the bottom channel enhances the flow rate and shear stress for endothelial cells, expanding applications to vascular biology along with immune cell recruitment.

A third offering, the Chip-A1™ Accessible Chip, has a lid to access a culture chamber that can accommodate ECM gel scaffolds up to 3 millimeters thick. This attribute enables the incorporation of stromal layers to create stratified epithelial tissue for exploring more complex biology, including tumor microenvironments, skin, and lung models. "The lid opens to expose cells to an aerosol or to directly apply a drug," said Ewart.

Organoids complement the technology. "When you combine organoids, for example, with the microfluidics and the stretch in the intestine models, you see greater in vivo relevance looking at gene expression profiles compared to organoids alone," discussed Ewart. Cells are polarized correctly, and functional transporters are expressed on the correct membranes at physiological levels. SEM demonstrates microvilli formation on mature, healthy, functional cells.

An Emulate partner at the Pasteur Institute showed that stretch was required to study Shigella infection. "The biological pathway to infect the cells becomes active in the Organ-Chip environment under stretch," explained Ewart. "This is yet another demonstration that our robust platform has value."



A human mature adipose tissue organoid containing adipocytes, fibroblast, and endo-thelial cells: *Red* = Actin, *Green* = Bodipy (specific for adipocytes) Blue = DAPI (nuclei), *Pink* = CD31 (endothelial cells). Cherry Biotech

Advancing accessibility

Organoids and associated culture instruments such as MPS continue to generate more scientific proof as they gain technology maturity. "The growing published knowledge base and recent regulatory incentives accelerate their overall acceptance," said Pierre Gaudriault, PhD, scientific chief business developer at **Cherry Biotech**.

The company provides 3D cell culture tools for preclinical drug development applications. The CubiX platform accurately and dynamically controls the microenvironment of complex 3D cell cultures like organoids and is fully compatible with live imaging and standard multiwell plates. It facilitates the mimicking of physiological and pathological culture conditions in vitro, including oxygen levels, media content and flow, drug conjugate concentration and delivery, and pH.

The Smartsphero plates, designed to control the size distribution and positioning of organoids, particularly for imaging applications, are based on a standard plate format for seamless integration into workflows. The plates utilize micro-patterned technology and contain microstructured hydrogels made of biocompatible polyethylene glycol for simple and consistent spheroid/organoid formation.

"In essence, we are converting the standard multiwell plate into an organ-on-a-chip," said Gaudriault. "The CubiX platform controls the microenvironment to allow development and culture of complex organoids, mimicking as closely as possible the human physiology."

The tools facilitate the maintenance of complex biological systems and cell types with a vascular interface and circulating

immune cells. Some prime applications for the agnostic technology focus on metabolic disease (e.g., obesity and type 2 diabetes) using human mature adipose tissue, plus oncology and inflammation studies. To empower their customers, Cherry Biotech's service capacity assists in validating proof-of-concept and protocols for specified applications.

Since its inception, the company has established close and strong relationships with KOLs to gain access to biological and practical expertise, forming large collaborations to develop and validate the technology.

To expand further, the field needs additional qualifications and statistically relevant data on the various devices on the market. "We have to be careful of our claims and communicate clearly what this technology can do and the benefits and limitations," said Gaudriault. "Alternative methodologies will not be a total substitute for in vivo models, only where needed and possible."

Predictive human models

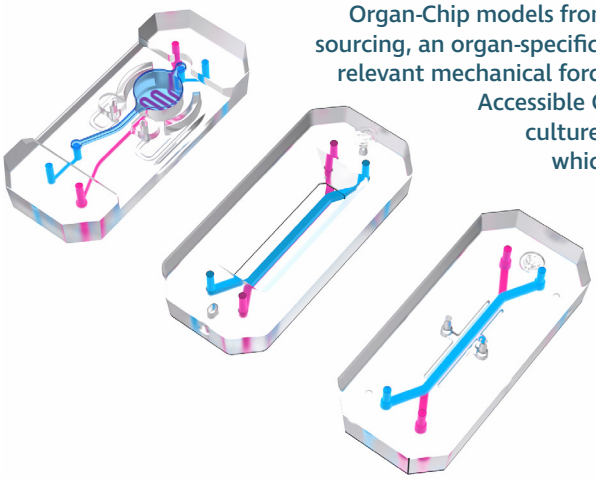
"MPS, also known as organ-on-a-chip (OOC) technology, encompass a wide range of predictive human models, each of which has specific applications in drug discovery and research," explained Emily Richardson, PhD, lead scientist at **CN Bio**.

A 2021 survey by the **IQ MPS Consortium** revealed that 20 out of 25 pharmaceutical and biotech companies had used or evaluated complex MPS technology in the last five years. In addition, a 2020 survey of investigative toxicologists in medium-large pharmaceutical companies showed that 20% already considered OOC a "game-changer," with another 60% anticipating it becoming one within five years for investigative toxicology.

CN Bio recently launched the PhysioMimix® DILI assay kit Human 24 to enable the prediction of complex, temporal, and immune-related hepatotoxicity. The kit uses primary human hepatocytes and Kupffer cells to mimic human liver function and immune responses, supporting detailed profiling of underlying mechanisms of DILI and key biomarker measurement.

"Generating human-relevant in vitro data enables better prediction of human outcomes whilst reducing reliance on animal models, improving the accuracy and translatability of preclinical studies," said Richardson.

"The time for MPS is now," emphasized Sung Lee, director of product management at CN Bio, "While rigorous validation studies are ongoing, the technology is demonstrating its potential. Standardizing protocols, robust QC measures, and consistent data analysis methods are crucial to ensure reproducibility and comparability of results. We are working towards establishing these standards and are already seeing compelling data emerge."



Organ-Chip models from Emulate combine human cell sourcing, an organ-specific microenvironment, and tissue-relevant mechanical forces. From left to right, Chip-A1™ Accessible Chip, which has a lid to access a culture chamber, Chip-R1™ Rigid Chip, which is made from minimally drug absorbent plastic, and Chip-S1® Stretchable Chip.

The industry hopes that validation studies will demonstrate the predictive power and reliability of MPS/OOC platforms across applications. This includes head-to-head comparisons with existing animal models and, where possible, clinical data. Specifically, the distribution of in vivo and clinical data from previous drug trials, particularly for failed drugs, can help OOC developers optimize their models and establish a robust solution for testing drug modalities with human-specific targets and pathways.

The continuation of open communication and collaboration between developers, pharmaceutical companies, regulatory agencies, and academic institutions is essential. Lee highlighted the importance of engaging with regulatory agencies in particular to help establish clear pathways for using MPS data in submissions.

Supplying organoids

Use cases of organoids are evolving. These diverse materials can represent a patient and/or disease and are thus potentially useful in target identification and characterization. Applications range from primary research up through late-stage preclinical tests.

"Organoids are additive in the new approach methodology (NAM) space," said Nikki Carter, commercial organoid innovation director at **Molecular Devices**. "Although animal models are still in use side-by-side with these alternative methodologies to understand the translatability, regulatory approvals are being made for NAMs in specific applications to replace animal use."

Consistently scaling these complex materials to meet demand is both crucial and challenging. Molecular Devices is

addressing the supply issue with their CellXpress.ai™ Automated Cell Culture System along with their other organoid expansion services. "We are recreating the 2D paradigm in terms of scaling and cryopreserving to ensure a consistent supply," clarified Carter. "We want to democratize the use of these important models by lowering accessibility barriers."

New models in the pipeline include healthy patient-derived organoids to evaluate GI toxicity and new iPSC models for DILI and cardiac toxicology. "Customers are also requesting different formats that we are working on validating," added Carter.

Organoid format is particularly pertinent for GI toxicology applications. Users want the diversity of cell types that come in a patient-derived organoid but also wish to fragment them for monolayer application in barrier assays. Fragmentation is complex. By their nature, organoids are self-assembling.

According to Carter, true market development comes from collaboration and a cohesive ecosystem. Molecular Devices takes an agnostic approach and works closely with HUB Organoids, which was recently acquired by the life science business of **Merck KGaA**, Darmstadt, Germany, to obtain patient-derived organoids along with organ-on-a-chip suppliers to advance applications.

Standardization, legislation, and regulatory guidance should help advance the field. "Model nomenclature, production, and characterization need to be standardized. Apple-to-apple comparisons are especially important for drug safety and toxicology testing," highlighted Carter. The cosmetic and personal care industry, which has minimized animal use, could also provide valuable learnings.

Analytical instrumentation

"Organ-on-a-chip can help simulate the mechanisms and responses of entire organs on the format of a chip. This can be particularly effective in spaces such as testing drug efficacy and seeing how new treatments interact with the blood-brain barrier. It gives you an advanced starting point," said Rebekah Sayers, manager of small molecule omics at **Sciex**.

Sayers pointed out that analytical instrumentation plays an important role in these innovative spaces, especially as each test may be unique. High-resolution mass spectrometers, such as the ZenoTOF 7600+ system, could facilitate novel sensitive metabolomic methods and identify lipid structures quickly. GEN