Shaping the Future of Predictive Toxicology: ATCC's HepatoXcell™ for Advanced MPS Platforms

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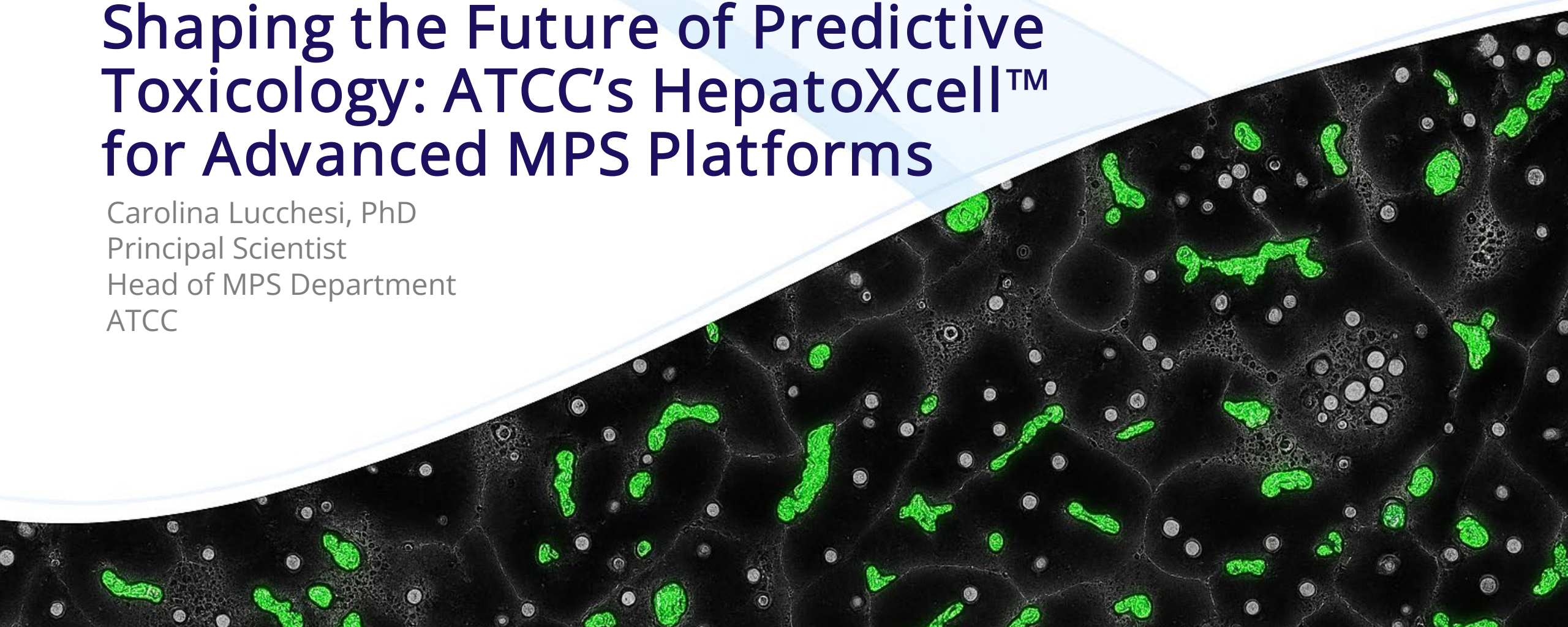
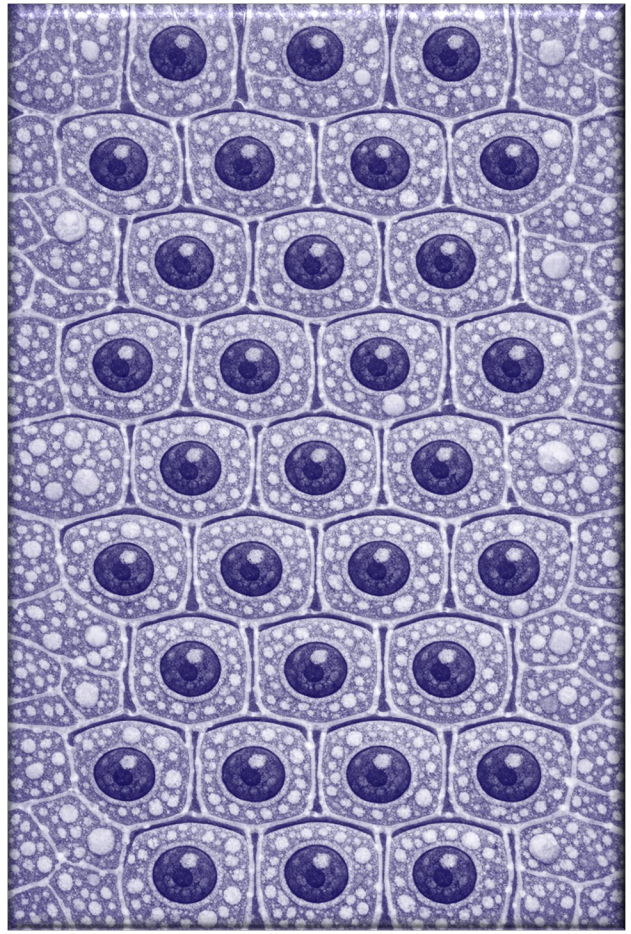
ATCC



Agenda



- Evolution of in vitro models
- Liver Relevance
- Microphysiological systems
- Hepatocytes characterization
- mRNA-LNP toxicity assessment

About Us

ATCC is a global leader in providing authenticated, high-quality biological resources and standards for industry, academia, and government.

- Founded in 1925, ATCC is a private, nonprofit, global biological resource center and standards organization that provides scientists with the biomaterials and resources they need to conduct critical life science research.
- World's trusted, premier biological materials resource and standards development organization:
  - 4,000+ cell lines
  - 80,000+ microorganisms
  - Genomic and synthetic nucleic acids
  - Media, sera, and reagents
  - Advanced cell models
  - Standards

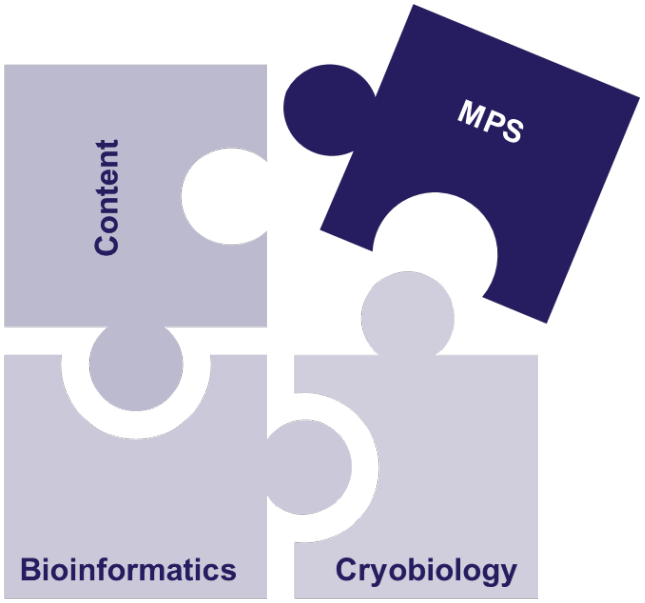
Modernization of the ATCC In Vitro Cell Model Portfolio

Past ATCC cell and micro collections were historically deposited by academic and other research scientists

Present ATCC R&D teams are actively developing new products to meet the needs of the scientific community

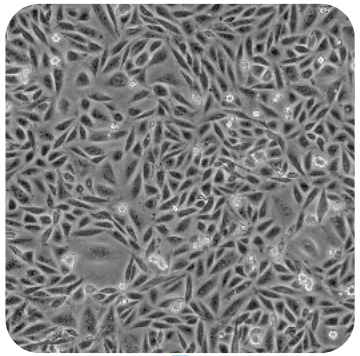
Ensuring the Future ATCC is investing in key technologies to ensure its products and services remain the definitive standards in biological research

ATCC LEADING BIOLOGICAL STANDARDS

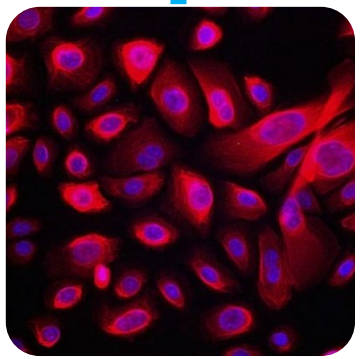


Research & Development

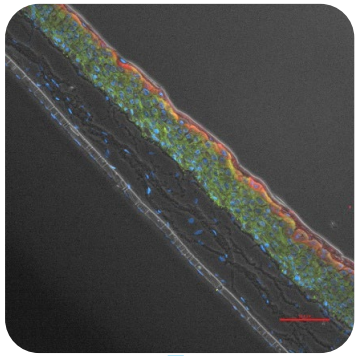
Evolution of In Vitro Cell Models



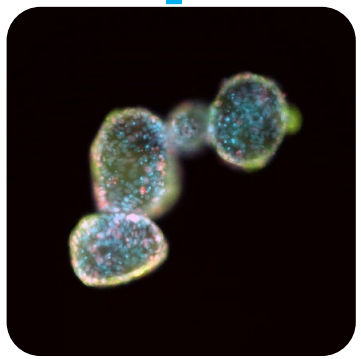
First generation
Continuous cell lines



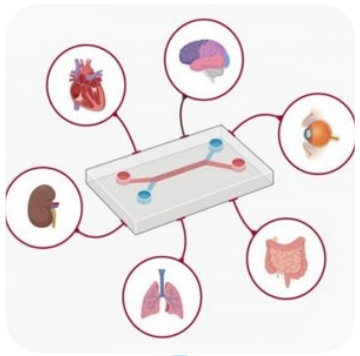
Second generation
Transfected cell lines
Reporter cell lines
Primary cells



Third generation
Immortalized primary cells
Gene-edited cell lines

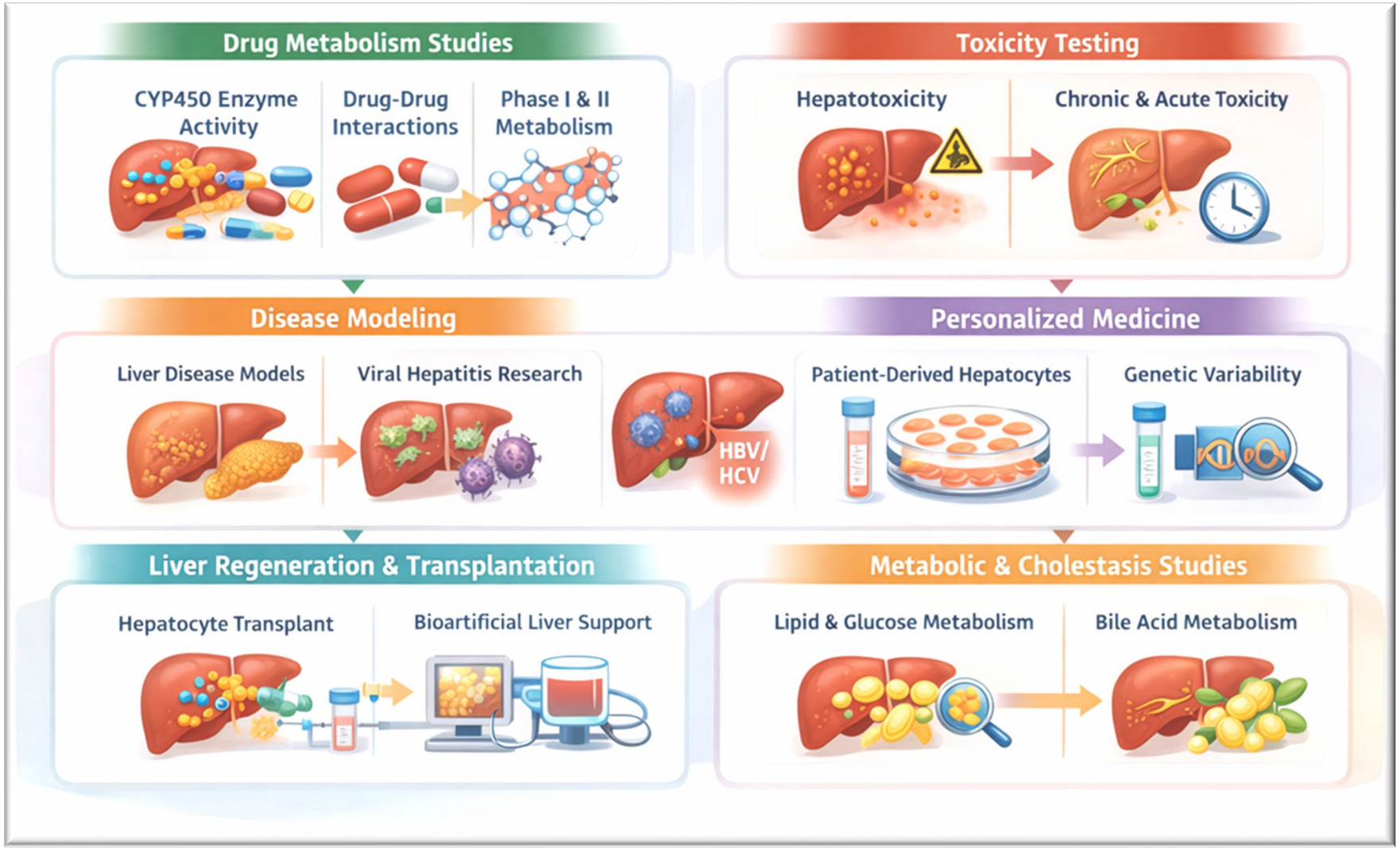


Fourth generation
3-D models
Organoids

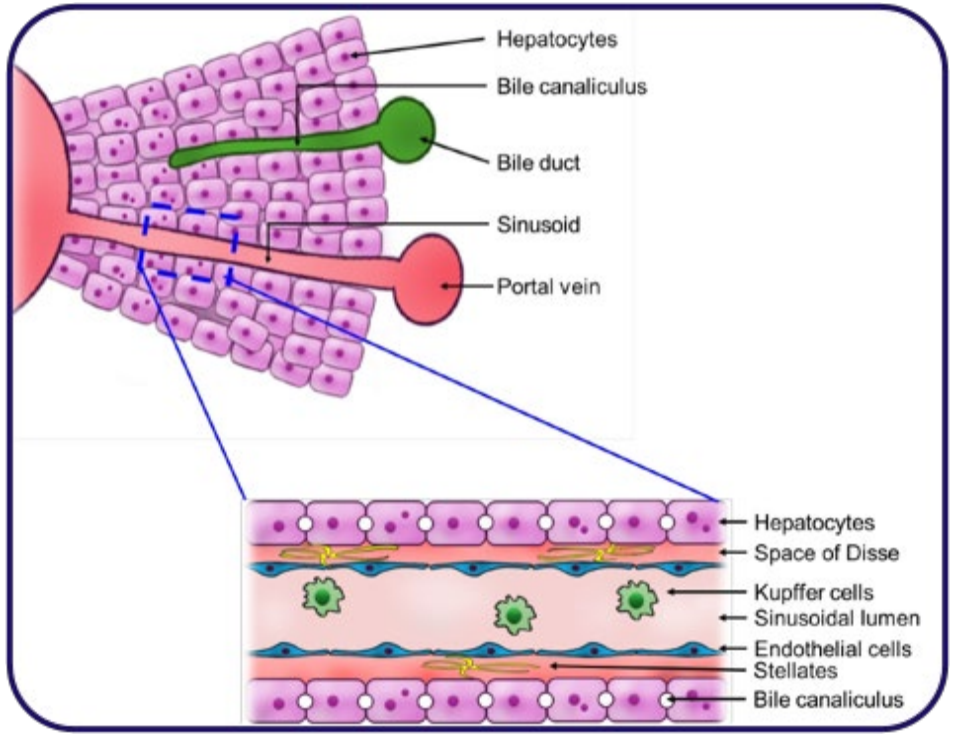


Fifth generation
Microphysiological systems
Organ-on-a-chip

Primary Hepatocytes Applications



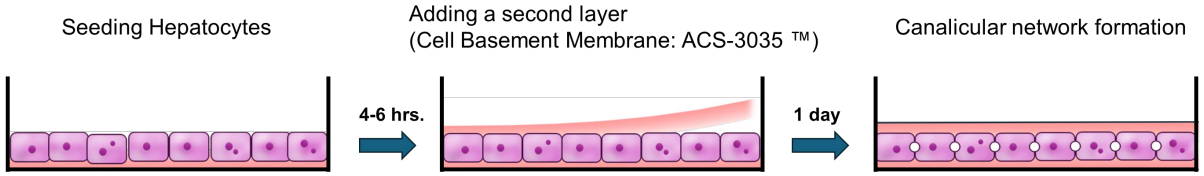
Liver Microphysiological Systems (MPS)



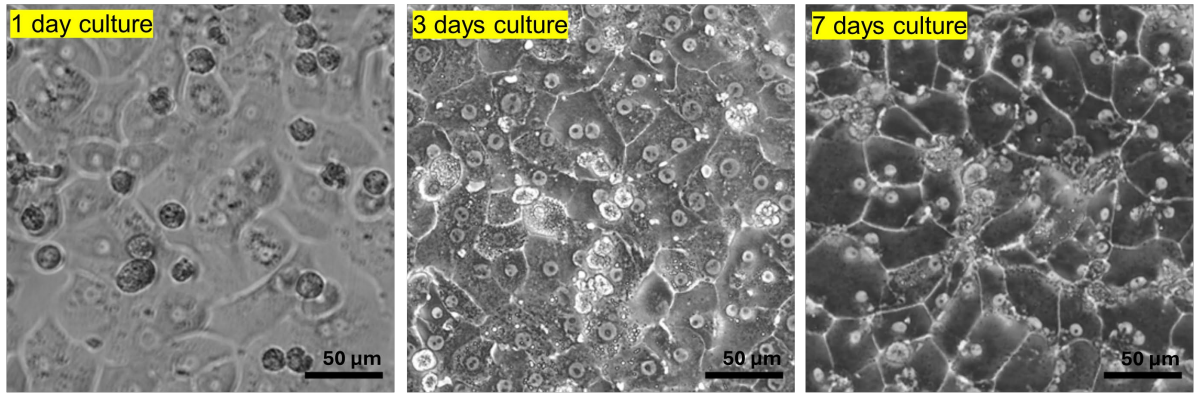
- **Human liver**: primary role is to metabolize drugs
- **Conventional human hepatocyte cultures**: often rapidly lose metabolic activity over time
- **Microphysiological systems:** integrate microfluidics to simulate the liver's dynamic microenvironment, enabling improved modeling of the human liver function over extended culture periods = **improved models of human liver functionality**

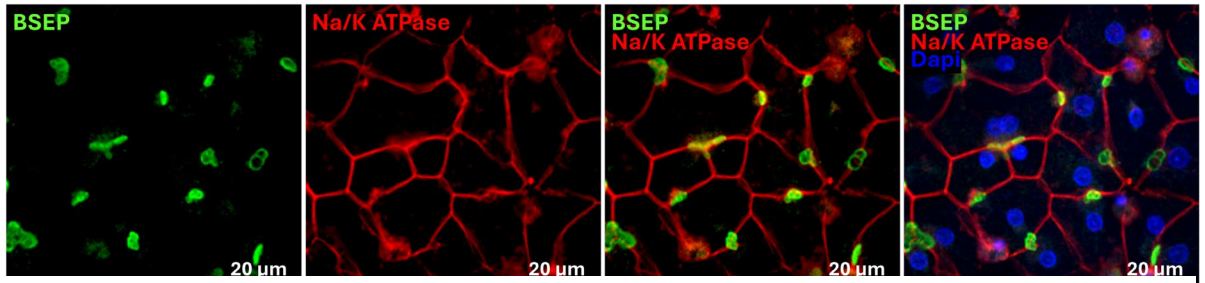
Regulatory agencies are requiring a shift from animal to human-based models
Ex: U.S. FDA's 'Roadmap for Reducing Animal Testing – New Approach Methodologies'

2-D Sandwich Culture as a Functional Baseline

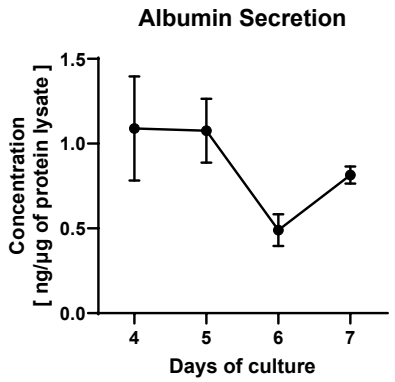


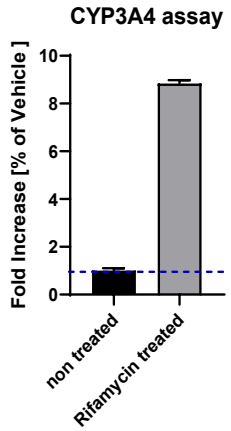
Sandwich-cultured HepatoXcell™ Pro



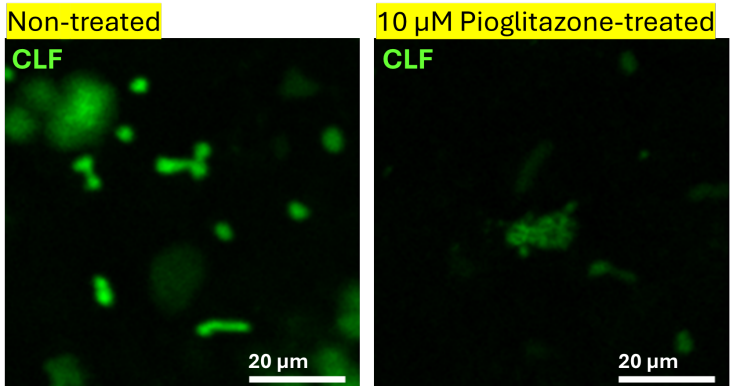


*BSEP (Bile Salt Export Pump); located on the canalicular membrane and transports bile salts. Na/K ATPase; located on the lateral membrane





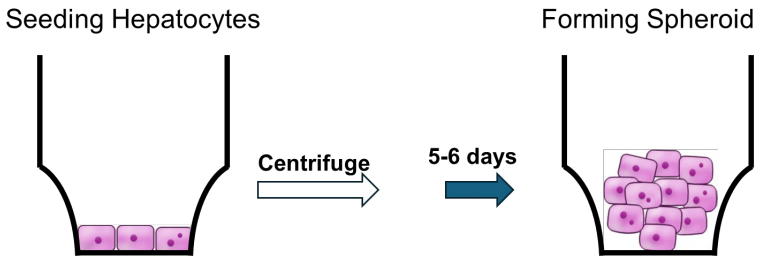
CLF efflux assay

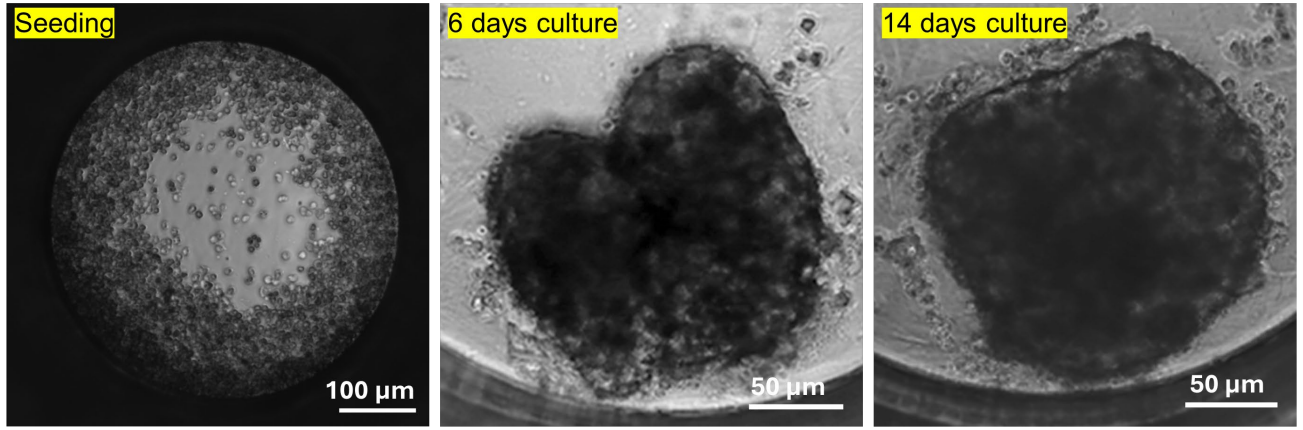


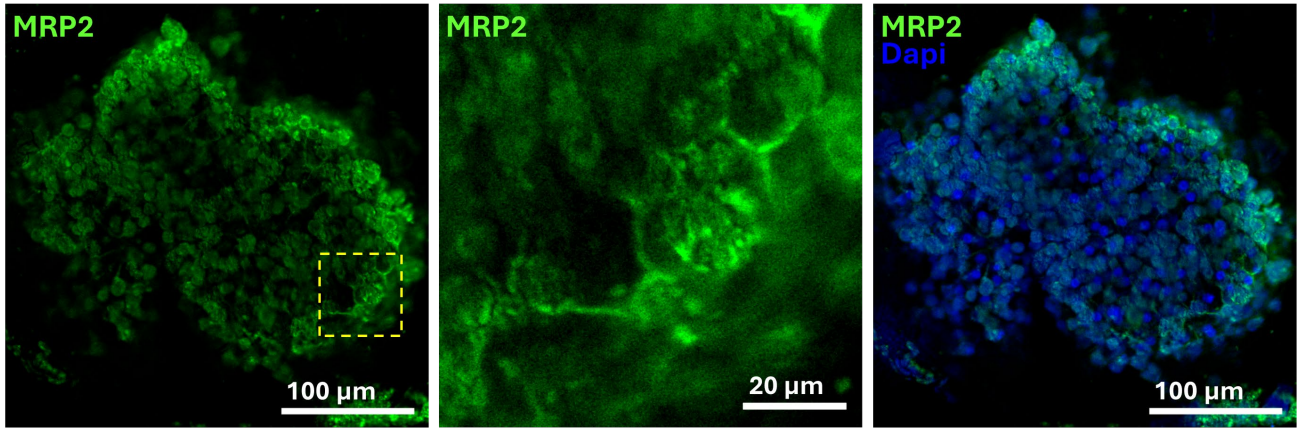
Hepatic transporters and functional efflux of bile canaliculi. *CLF (Cholyl-Lysyl-Fluorescein); fluorescent bile acid analog

Sandwich culture supports polarized hepatocytes with functional canaliculi, enabling transporter assays (e.g., CLF efflux).

HepatoXcell™ Pro Forms Matrix-free 3-D Spheroids

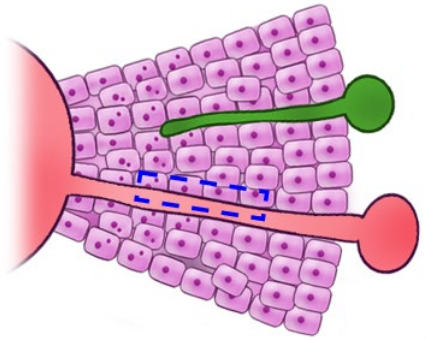


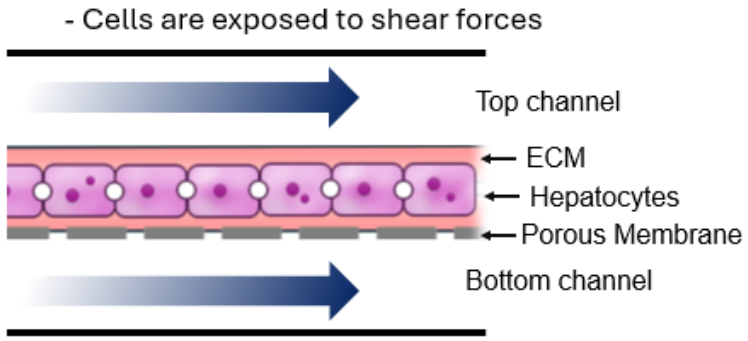




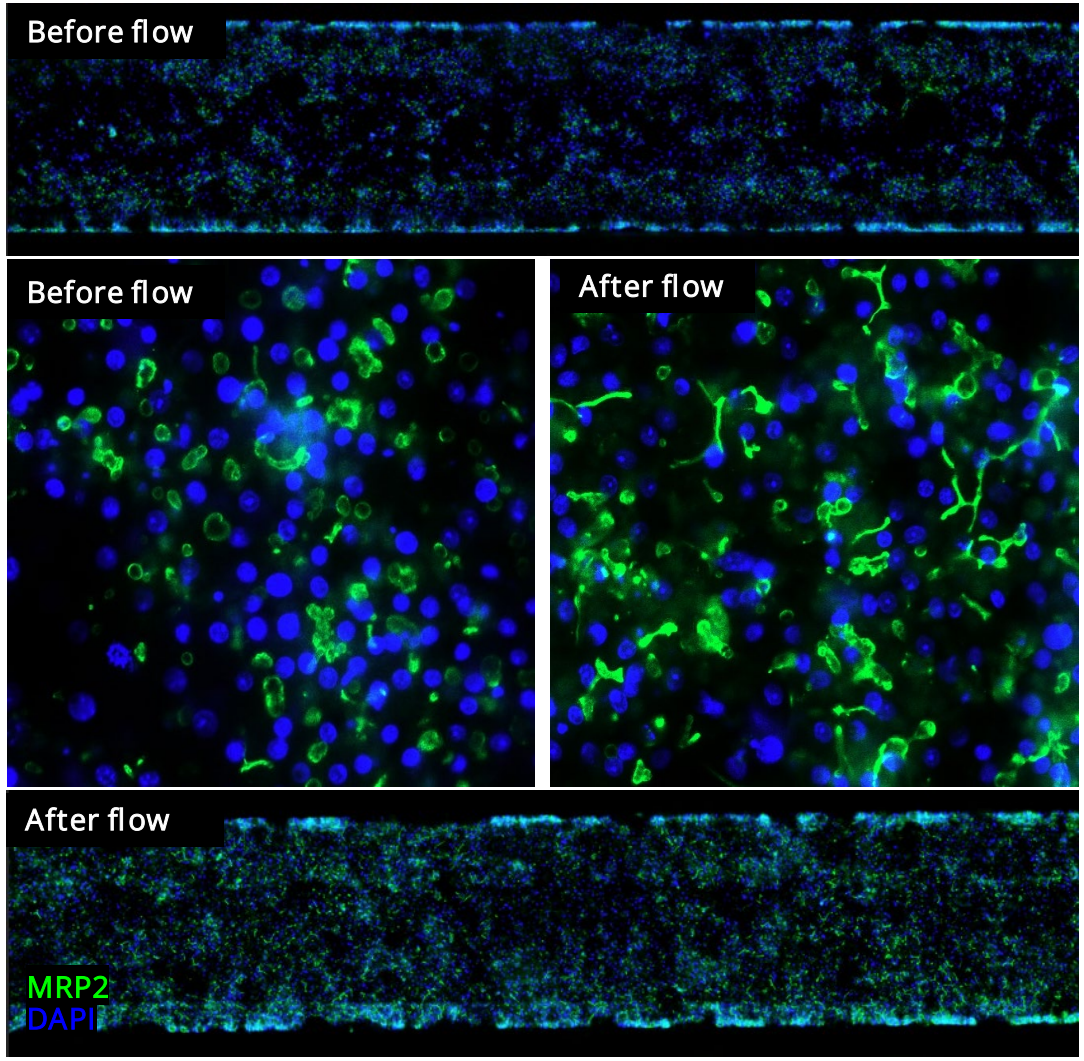
Hepatocytes self-assemble into 3-D spheroids without added matrix, supporting longer culture and 3-D workflows.

HepatoXcell™ Pro on Fluidic System

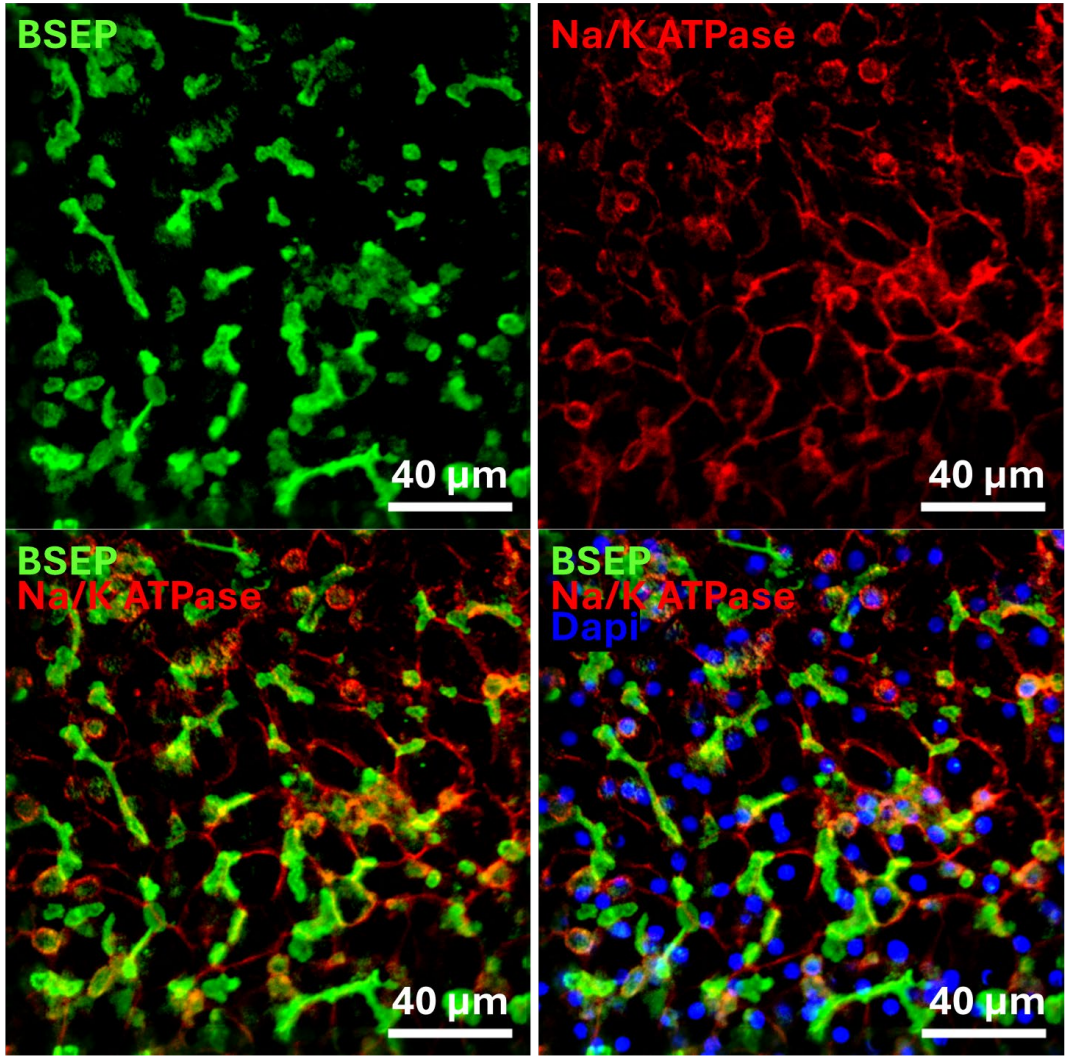




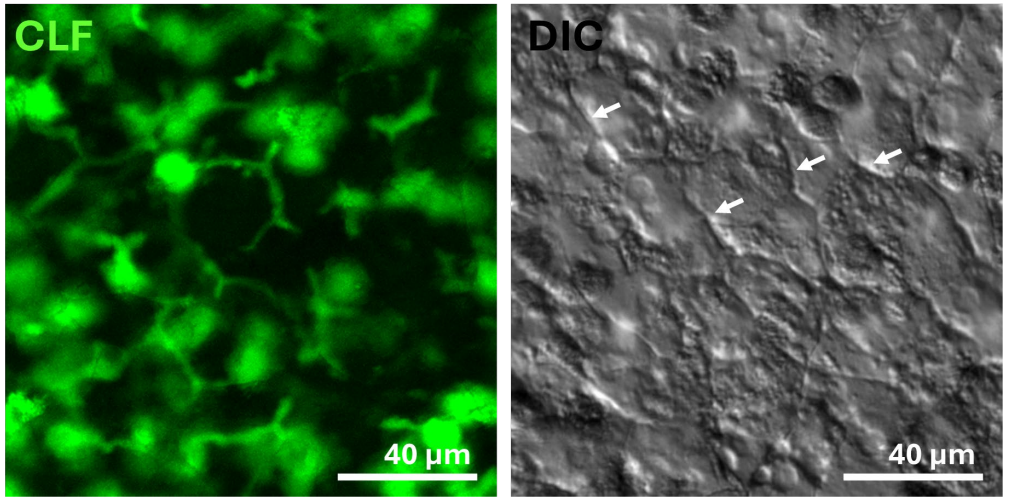
Multidrug Resistance-Associated Protein (MRP2) has expression pronounced under fluidic conditions



Fluidic System with HepatoXcell™ Pro



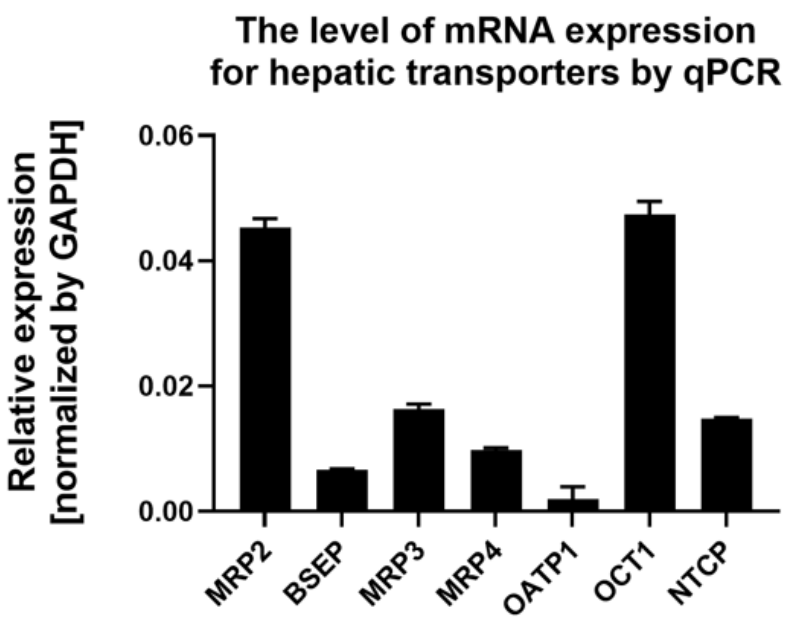
CLF assay to measure bile acid efflux



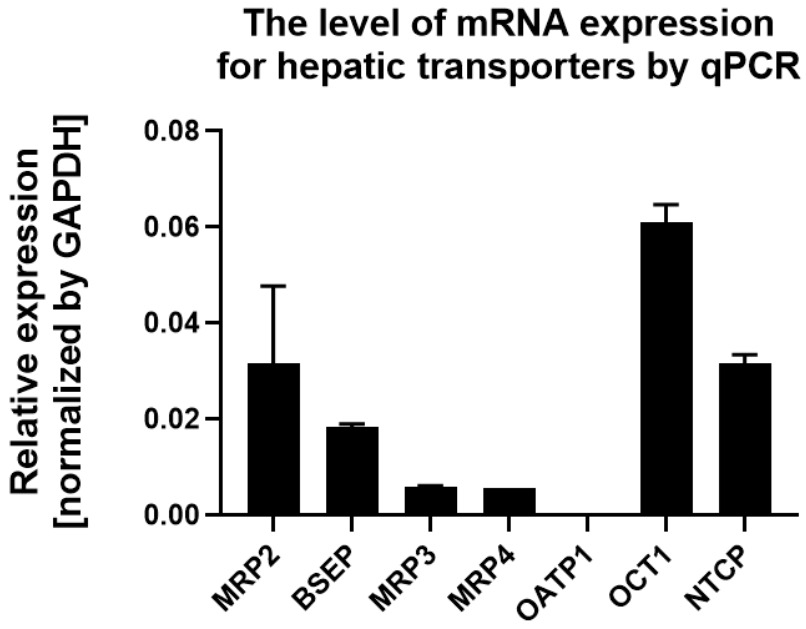
CLF bile acid efflux demonstrates functional canalicular transport, supporting the physiological relevance of this liver-on-a-chip transporter-mediated risk assessment

Gene Expression of HepatoXcell™ Pro Across Systems

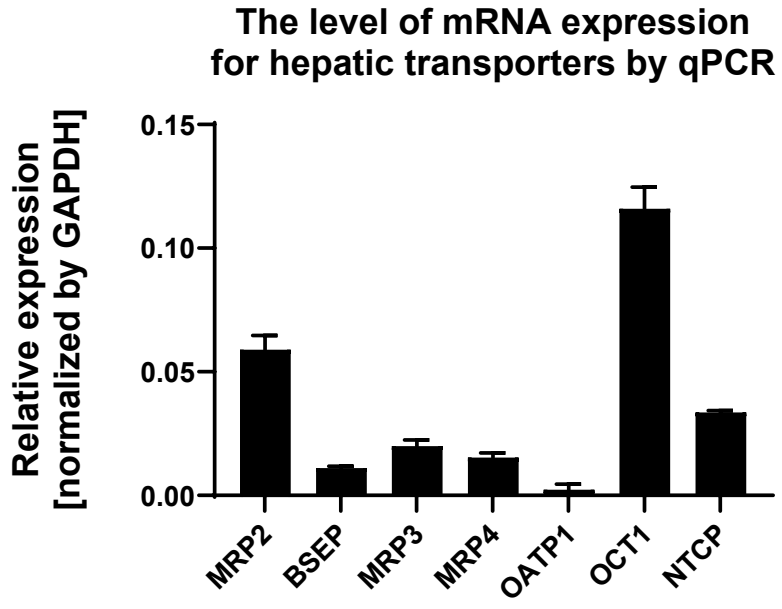
Spheroid Culture



Fluidic Culture



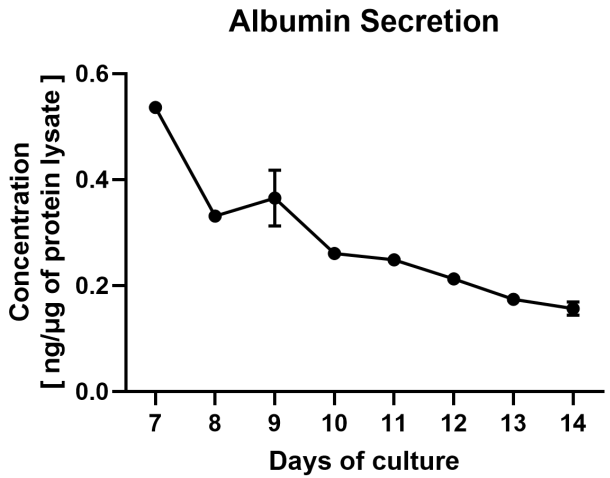
Fluidic Scaffold Culture



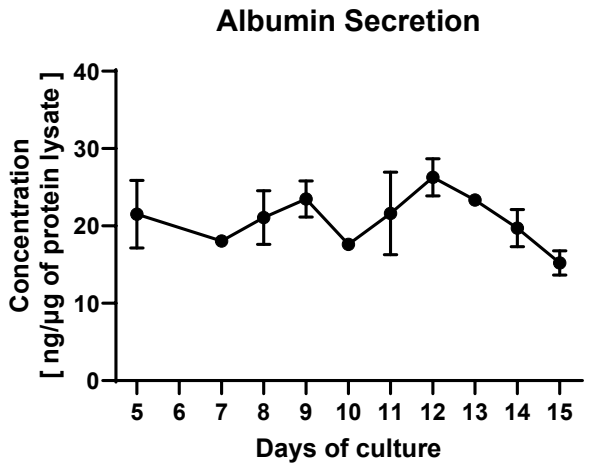
The gene expression demonstrates the presence of the main hepatic transporters.

Albumin Secretion of HepatoXcell™ Pro Across Systems

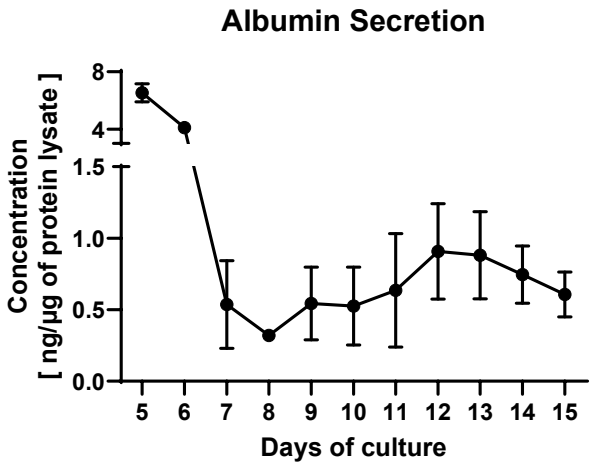
Spheroid Culture



Fluidic Culture



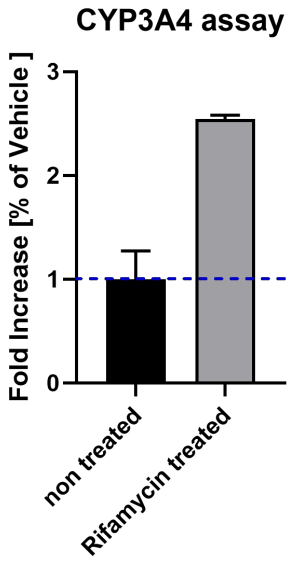
Fluidic Scaffold Culture



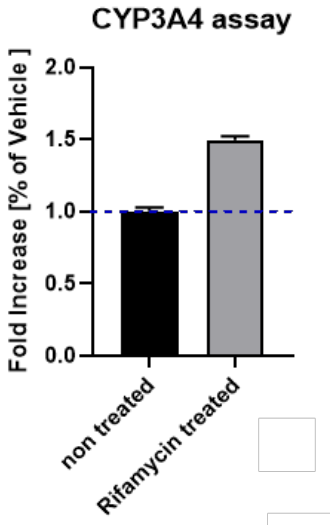
Albumin secretion across platforms. Fluidic systems maintain secretion for 2 weeks.

Enzymatic activity of HepatoXcell™ Pro Across Systems

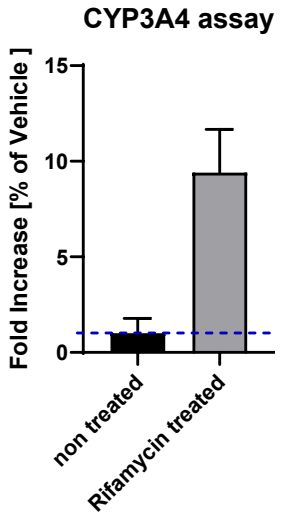
Spheroid Culture



Fluidic Culture



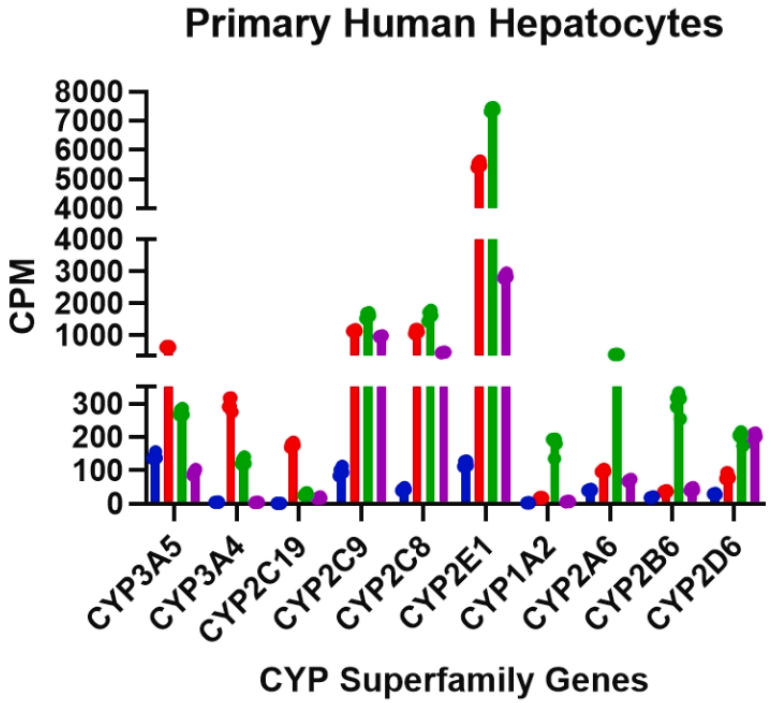
Fluidic Scaffold Culture

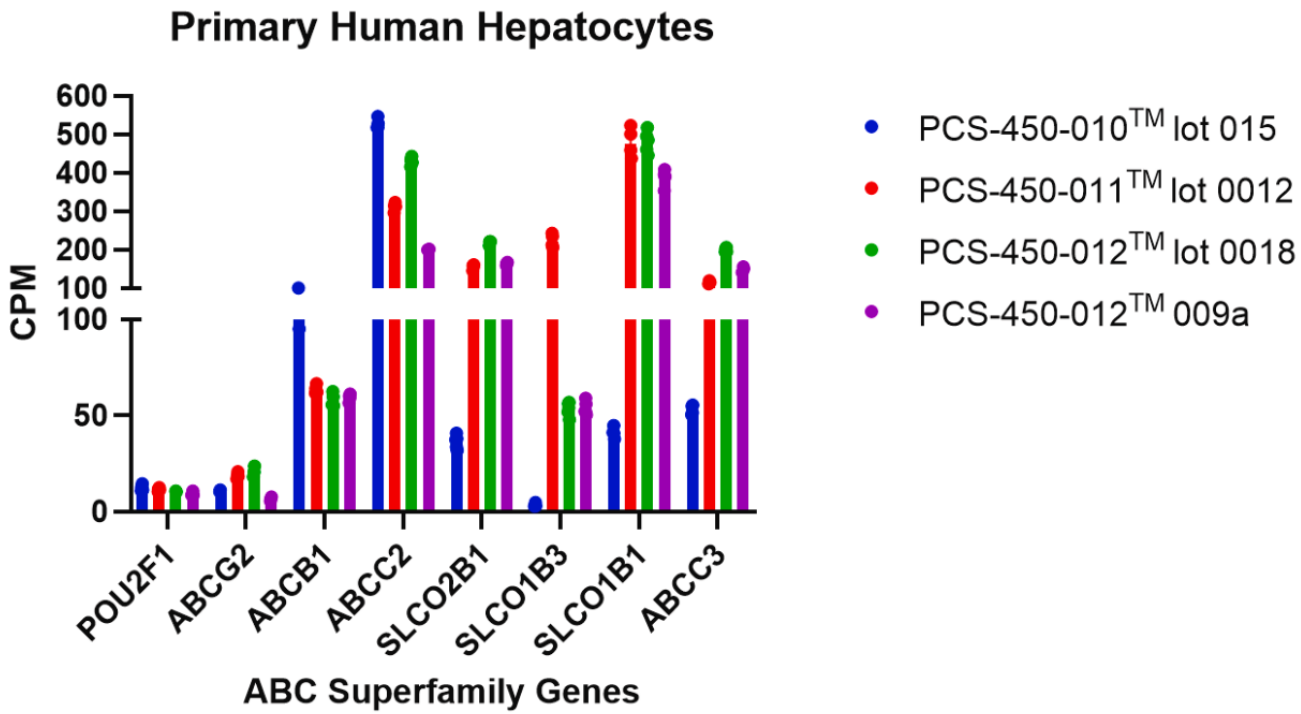


HepatoXcell™ demonstrates improved functionality in the fluidic system.

HepatoXcell™ Transcriptomic

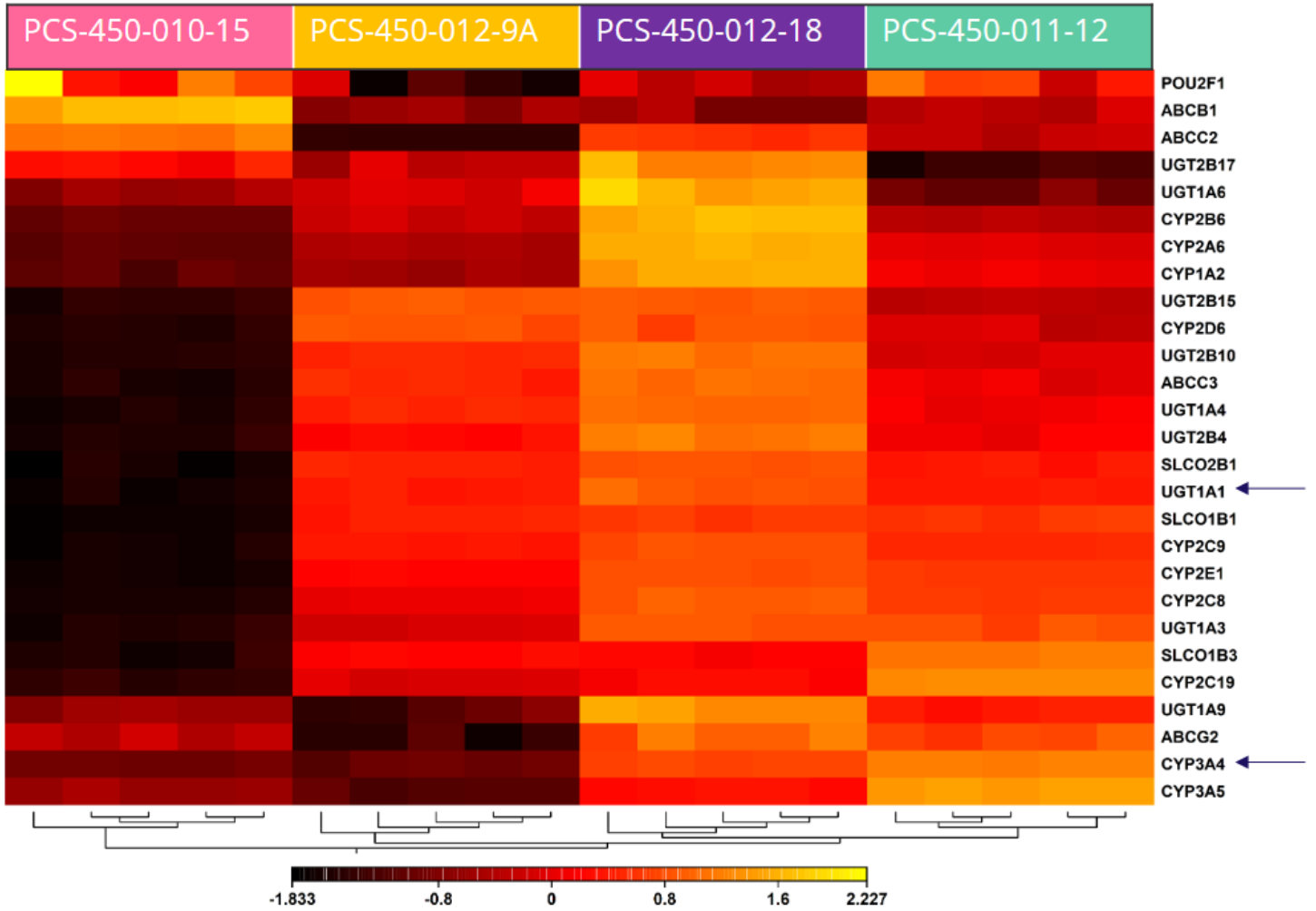
- Collaboration with the bioinformatics team and Precision Quantomics
- Transcriptome and exome data available for each lot





Gene expression profiling of key cytochrome P450 enzymes and transporters across hepatocyte lots

HepatoXcell™ Applications



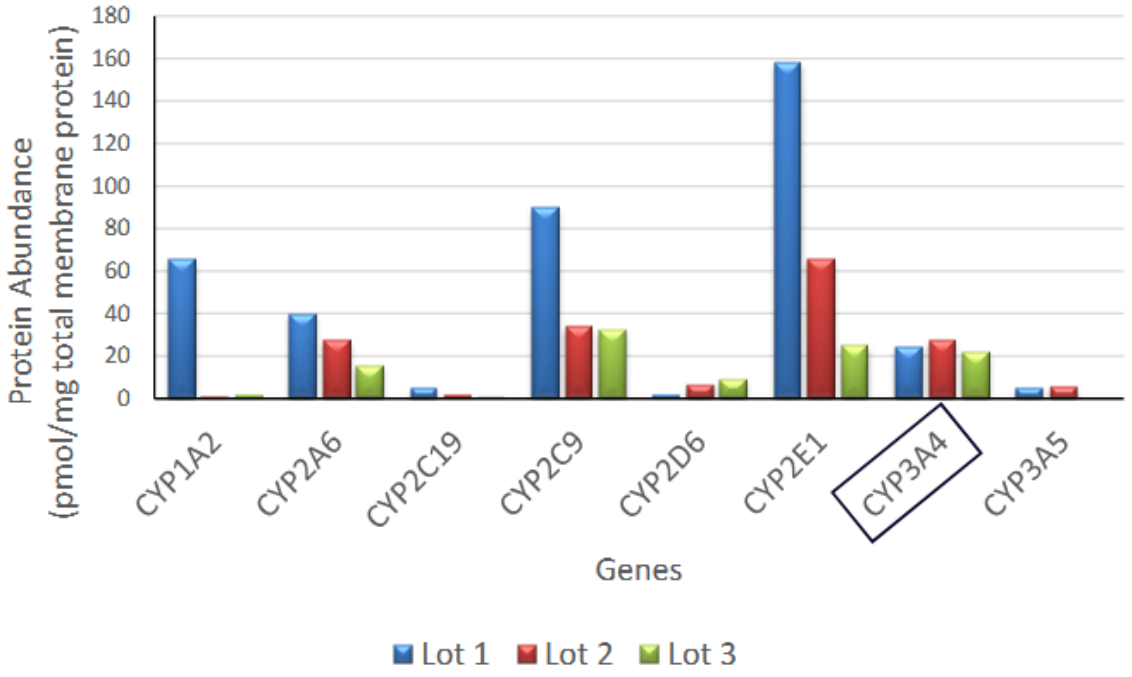
PHH Lot

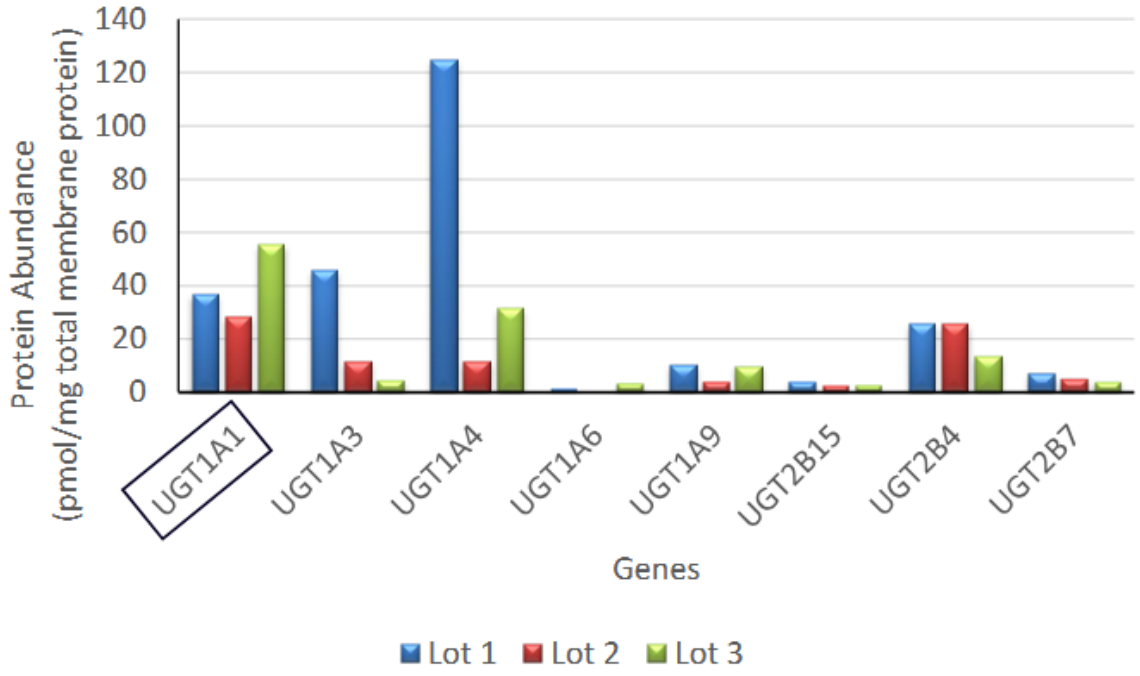
- PCS-450-010-15
- PCS-450-012-9A
- PCS-450-012-18
- PCS-450-011-12

Cross-donor comparison of key gene expression

HepatoXcell™ Applications

Donor-specific differences in metabolizing enzyme abundance





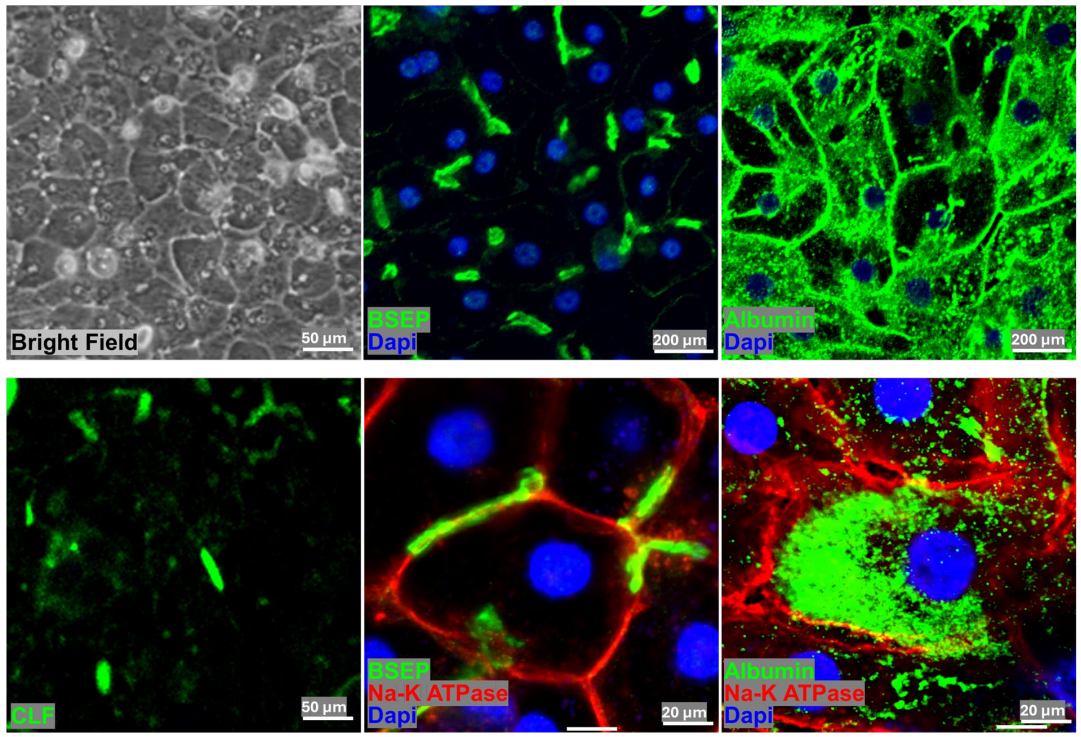
HepatoXcell™ Catalog #	Lot #
PCS-450-011-0012	1
PCS-450-011-0027	2
PCS-450-011-0037	3

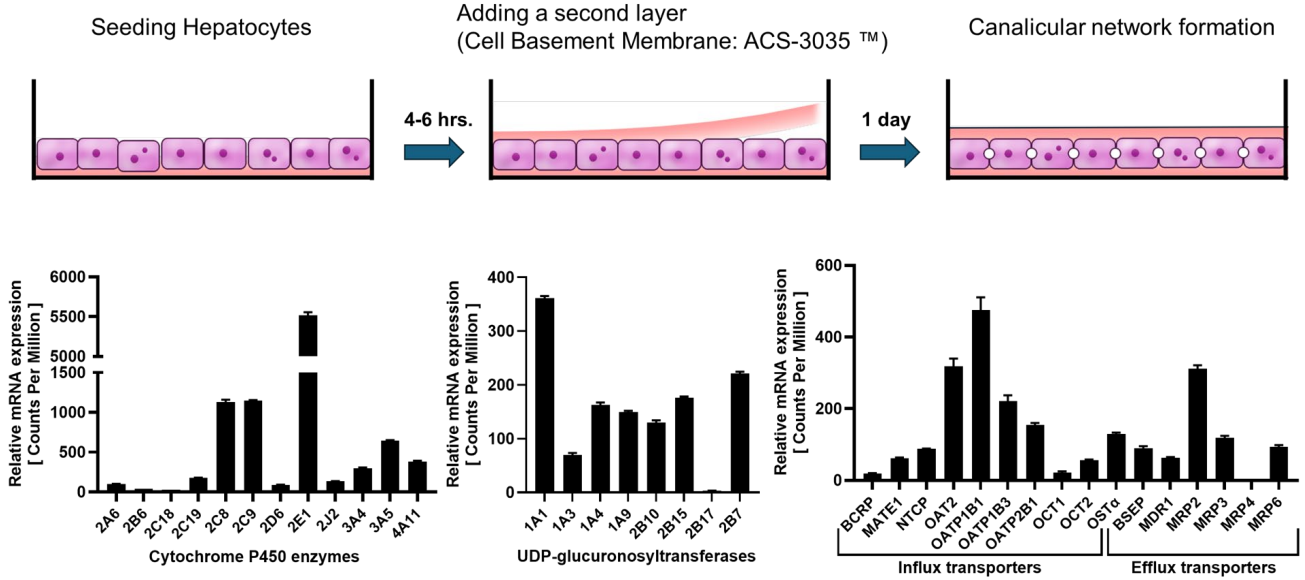
Protein expression profiling of key cytochrome P450 enzymes and transporters across hepatocyte lots

Evaluating mRNA Delivery Performance and Hepatotoxicity in Liver-Chips

Qualification in 2-D Sandwich Culture

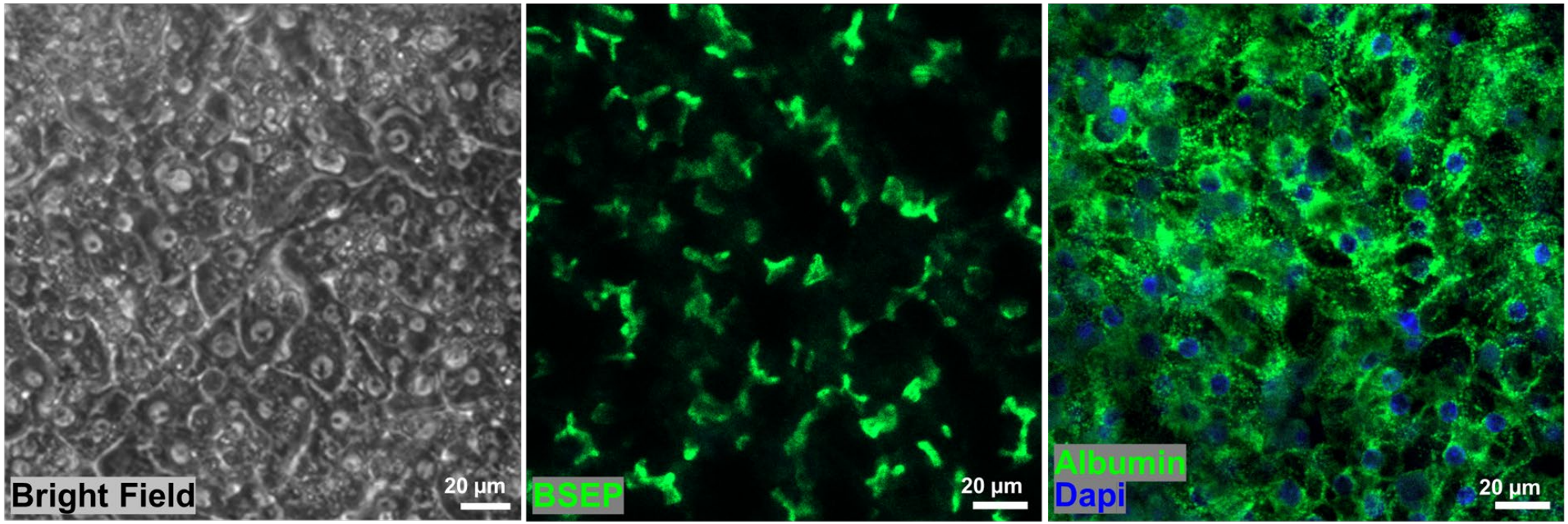
Stable function and transporter activity





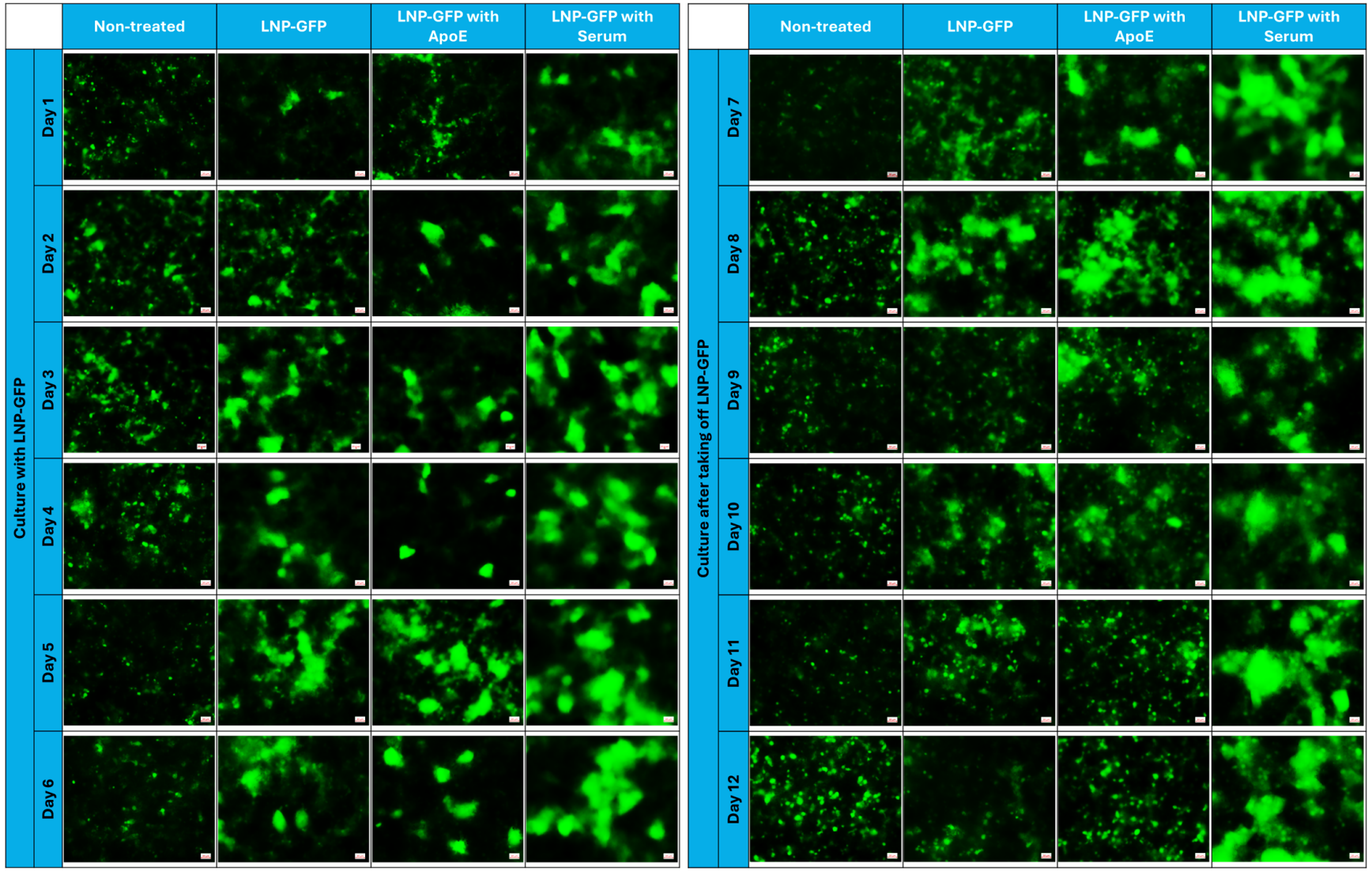
HepatoXcell™ demonstrates stable hepatic function and transporter activity in 2-D sandwich culture, supporting its translational readiness for predictive toxicology and MPS integration.

Flow-Enabled Maturation of HepatoXcell™ in Liver-Chip



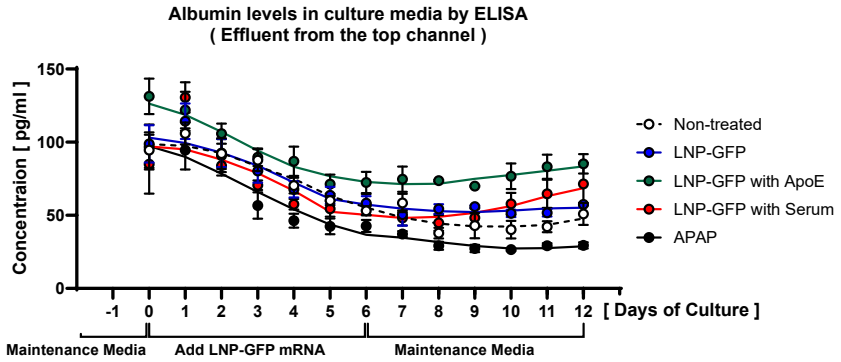
Microfluidic perfusion promotes polarization and maturation, enabling stable liver-chip performance over extended culture (20 days)

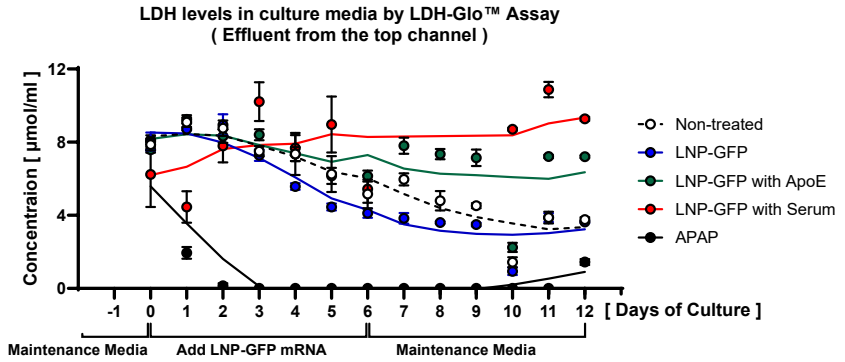
Efficient, Media-Dependent LNP-mRNA Expression in Liver-Chip

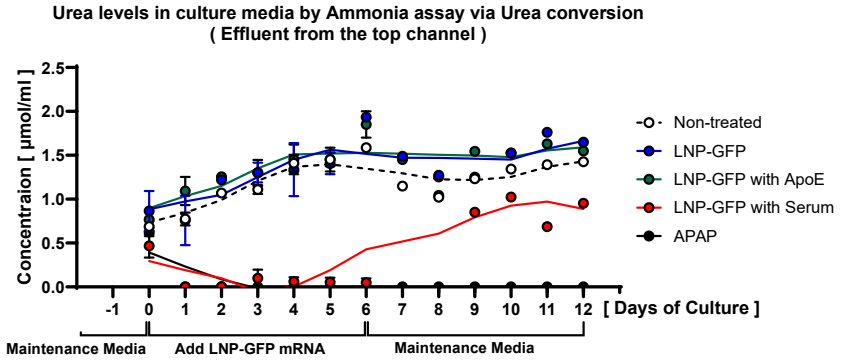


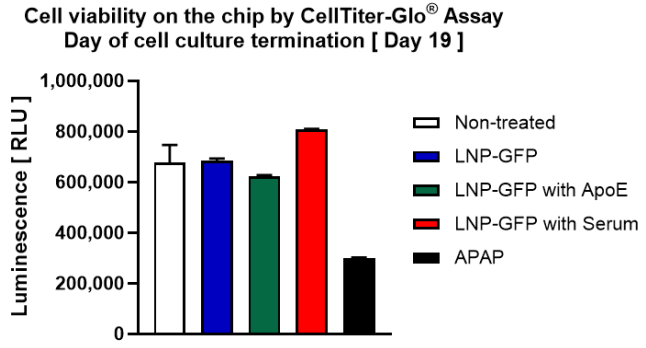
LNP-mRNA delivery drives robust, sustained expression in liver-chips with clear media dependence, supporting delivery and safety evaluation.

Minimal Hepatotoxicity Following LNP-mRNA Exposure in Liver-Chip









LNP-mRNA treatment shows no detectable hepatotoxicity in the liver-chip, with stable albumin secretion, reversible urea changes, and no adverse cytotoxicity signals, supporting the platform's suitability for RNA therapeutic safety assessment.

Summary and Outlook

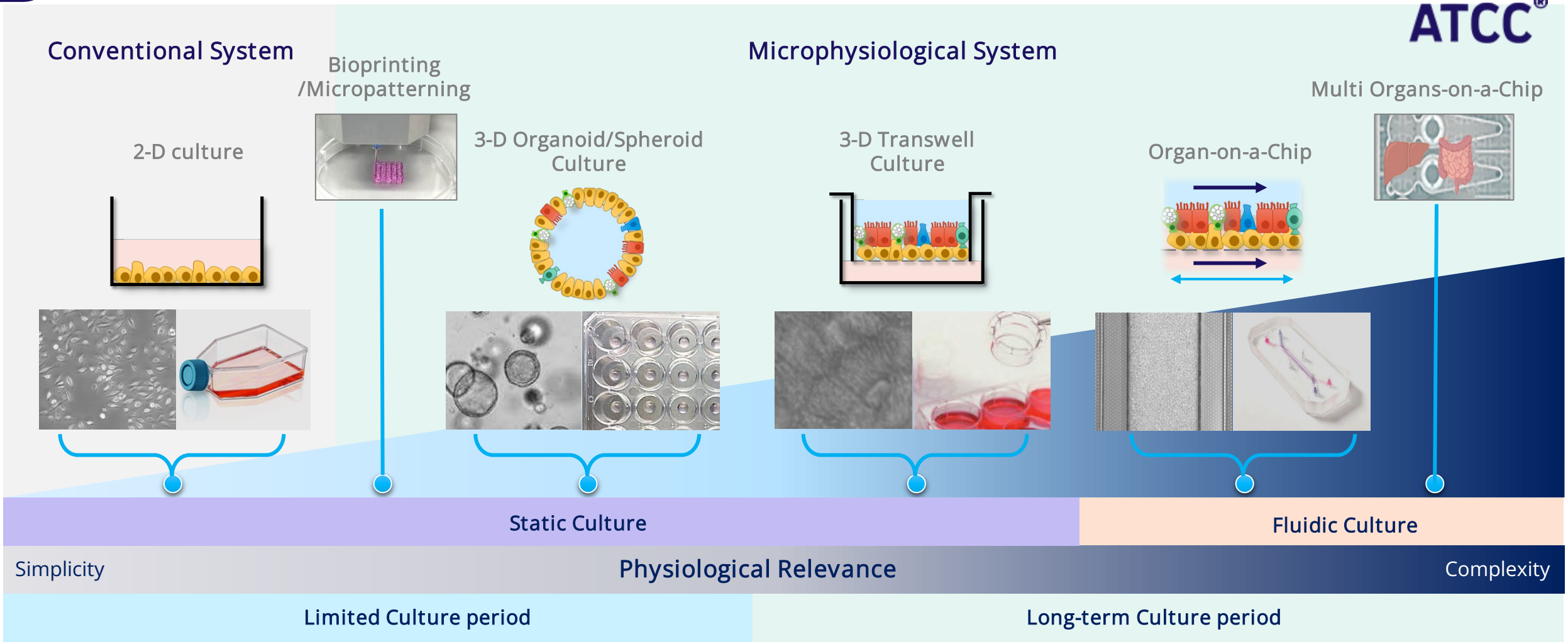
Key Takeaways

- HepatoXcell™ Pro maintains a hepatic phenotype under perfusion, preserving polarity, canalicular networks, and metabolic function.
- Stable long-term performance in liver-chip supports mechanistic mRNA delivery assessment.
- Efficient, media-dependent LNP-mRNA expression occurs with minimal hepatotoxicity signals.

Program Outlook

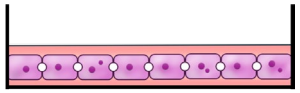
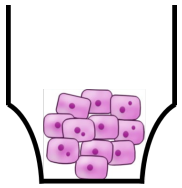
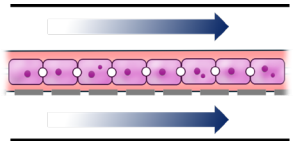
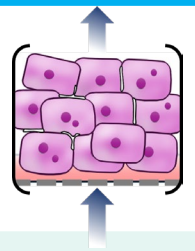
- Expand assay panels for drug-induced liver injury and disease-relevant toxicity.
- Integrate non-parenchymal co-cultures to increase physiological complexity.
- Develop dual- and multi-organ systems (e.g., liver–gut, liver–lung) to enhance translational relevance.

HepatoXcell™ Applied in Various In Vitro Cultures Systems



MPS technology integrates microfluidics to simulate the physiological dynamic microenvironment, which includes blood flow and complex cell-cell interactions that replicate the 3-D tissue structure and support culture for extended periods while preserving the functional activity

Guide of Potential Uses for HepatoXcell™ Pro

Culture System	Sandwich culture	Spheroid culture	Fluidic culture	Fluidic scaffold culture
Diagram of System				
Types of System	Transitional culture System	Microphysiological System		
Physiological relevance	Medium	Medium	High (+++++)	High (+++)
Media flow	No	No	Yes	Yes
Culture period	7 days	~ 1 month	Over a month	
Co-culture	N/A	N/A	Yes, with Non-Parenchymal Cells	
Polarization/ Canaliculi formation	Yes	Yes	Yes	Yes
Expression of transporters	Yes	Yes	Yes	Yes
Metabolic Activity	Yes (++)	Yes (++)	Yes (+++++)	Yes (+++)
Initial seeding	$4x10^5$ cells/well (24 well)	$0.15x10^4$ cells/well (96 well)	$\sim 1.2x10^5$ cells/chip	$6x10^5$ cells/chip
Assay friendly	++	+	+++++	+++

Meet our Experts

ATCC Booth #1143

Tiny Tox Talk

Mini Organs, Major Insights: Toxicology Goes 3-D

- **Presenter:** Carolina Lucchesi, PhD
- **Date:** Wednesday, March 25, 11:20–11:40 AM
- **Location:** Tiny Tox Theater (close to poster boards D264–D359 in the Blue poster section)

Poster

Human Liver-on-a-Chip System for Enhanced Mechanism-Based Toxicity Screening

- **Presenter:** Carolina Lucchesi, PhD
- **Date:** Tuesday, March 24, 9:15–11:45 AM
- **Board:** H618, **Poster:** 4136

Poster

ATCC's Human-Relevant Cancer Models for Mechanism-Based Toxicity and Drug Response Profiling

- **Presenter:** Ajeet Singh, PhD
- **Date:** Tuesday, March 24, 9:15–11:45 AM
- **Board:** H622, **Poster:** 4140

Poster

Generation of a Novel Immortalized Human Corneal Epithelial Cell Line for Reliable In Vitro Ocular Toxicity Studies

- **Presenter:** Xiangshan Zhao, PhD
- **Date:** Tuesday, March 24, 9:15–11:45 AM
- **Board:** M839, **Poster:** 4340

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Questions