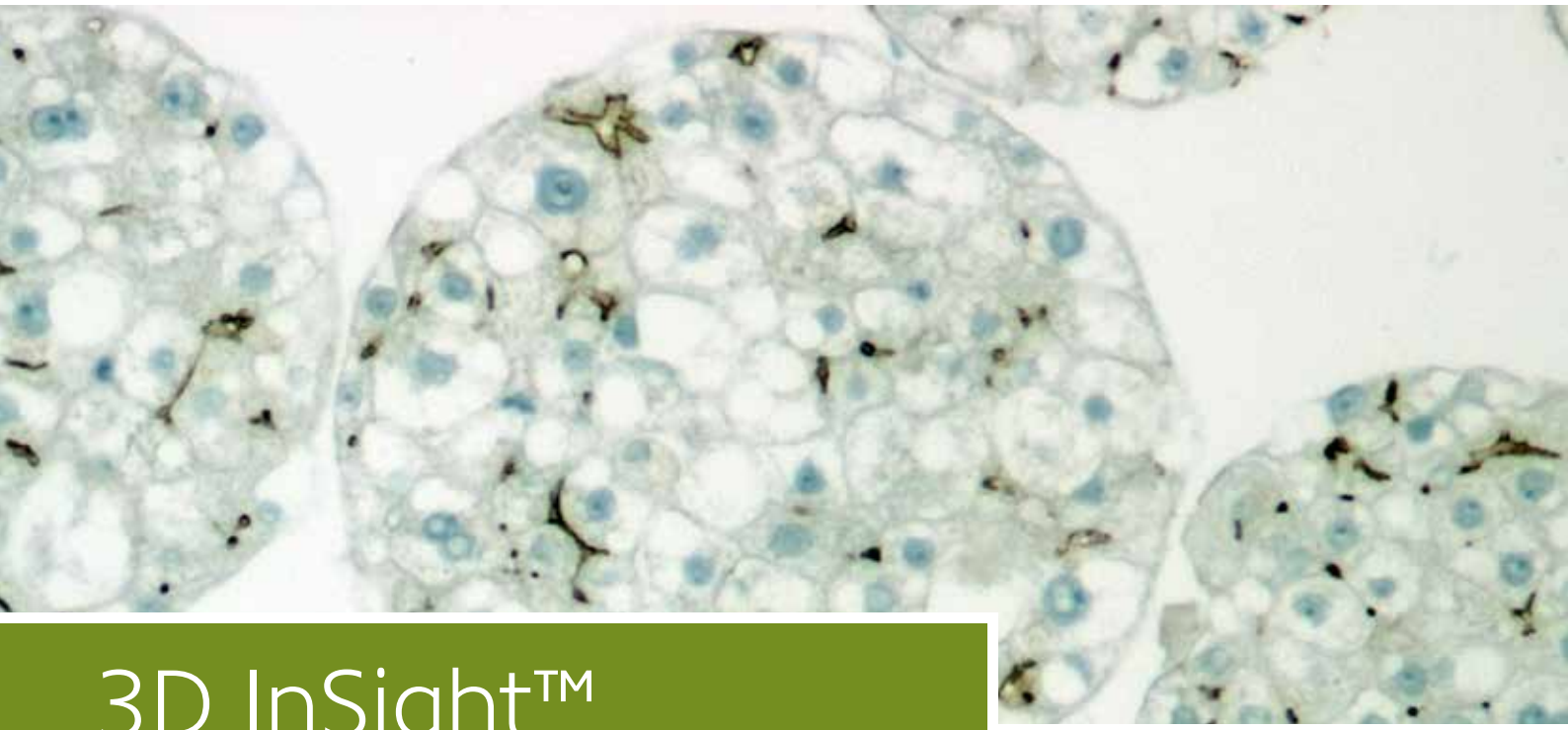
insphero
Biomimetic Microtissue Technology



3D InSight™
Liver Microtissues

Organotypic 3D Liver Models for ADME-Tox & DMPK

- Human Liver
- Rat Liver
- HepG2
- Liver Toxicology Services

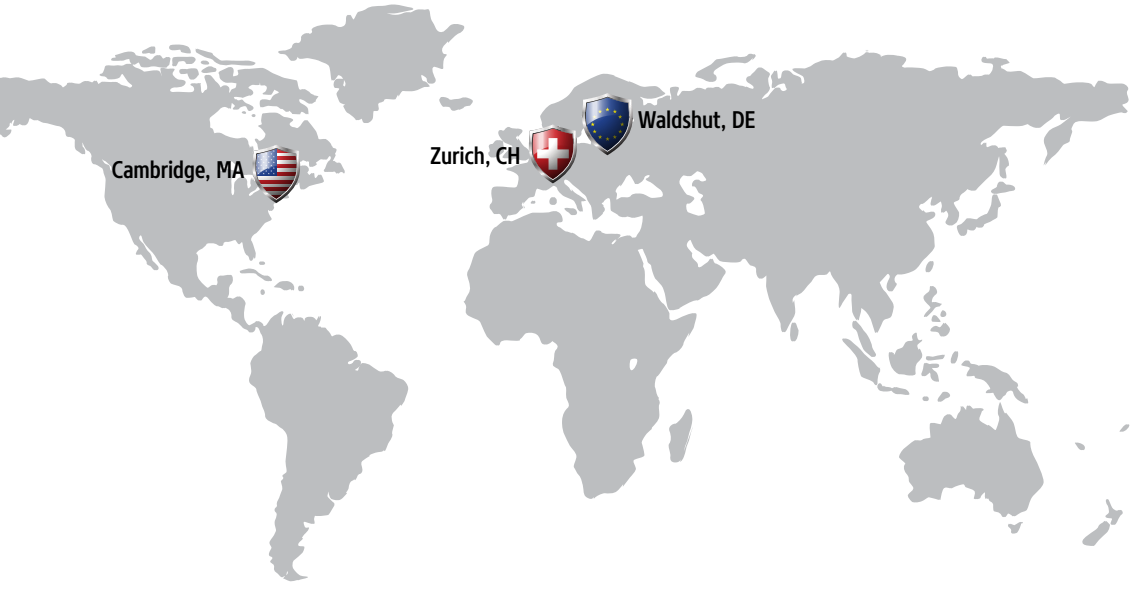
inSphero
Biomimetic Microtissue Technology

A World of Discovery in Every Hanging Drop

InSphero is the leading supplier of organotypic, biological *in vitro* 3D microtissues for highly predictive drug testing. Headquartered in Zurich, Switzerland with subsidiaries in the USA and Germany, we currently count all of the top 15 global pharmaceutical and cosmetics companies as our customers. Based on our patented, fully automatable hanging-drop technology and proprietary processes for cell conditioning, InSphero offers a broad range of highly functional, uniform and long-living 3D InSight™ Microtissues.

Delivering a more biologically relevant model for *in vitro* applications, we are the first and largest provider of assay-ready spheroids and 3D-focused contract research services for toxicology, DMPK, and efficacy studies. We have been recognized for our scientific and commercial achievements with a number of national and international awards, and are certified to the ISO 9001:2008 standard for our Quality Management System.

Upgrade Your Cell-based Assays to 3D Today!



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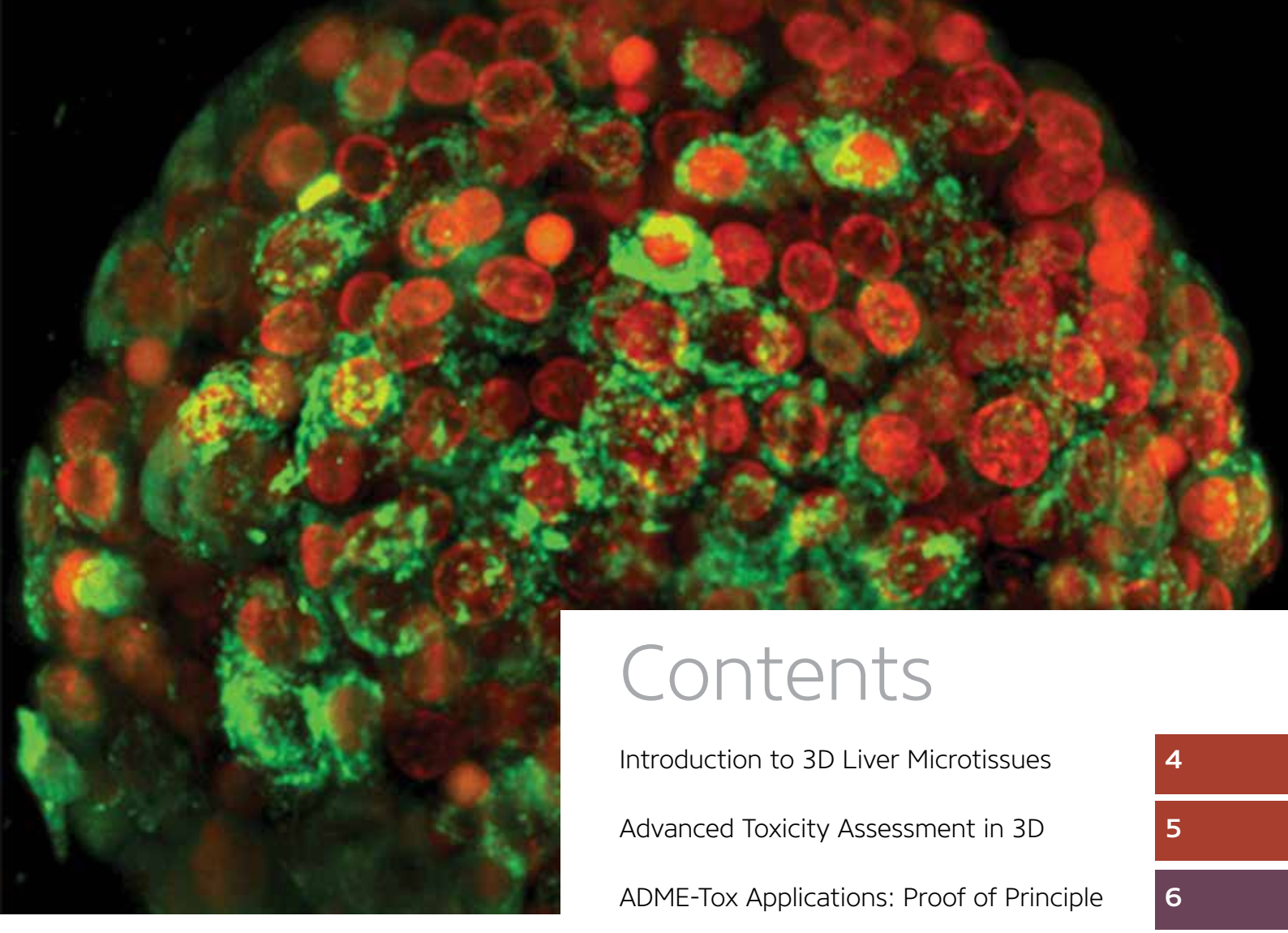

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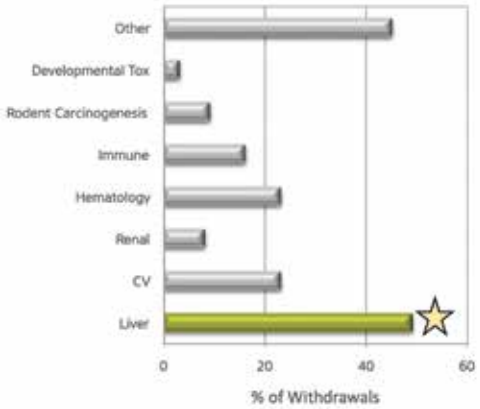
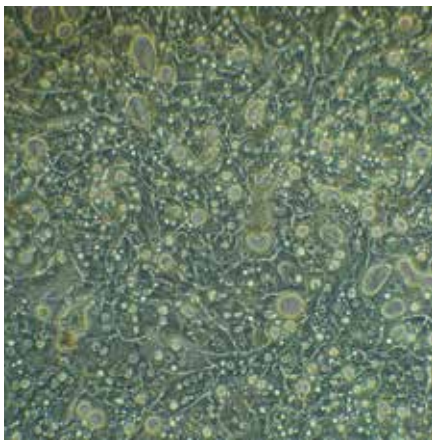
Filling the Need for More Predictive In Vitro Liver Models

"...conventional (2D) in vitro hepatic model systems...are less than ideal for longer term toxicity evaluations and elucidation of key cellular and molecular events involved in primary and secondary adaptation to chemical exposure..."

LeCluyse et al. Crit Rev Toxicol. (2012) Jul;42(6):501-48.

50% of Drug Attrition in Development is DILI Related





Common Failures of 2D Liver Model Systems

- Short-term viability
- Rapid loss of liver-specific morphology & phenotype
- Lack complex extracellular matrix

3D InSight™ **Liver Microtissues**

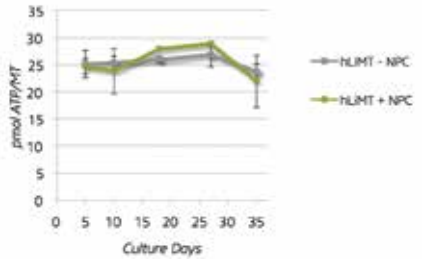
Defining a New Standard

Increased Long-term Viability

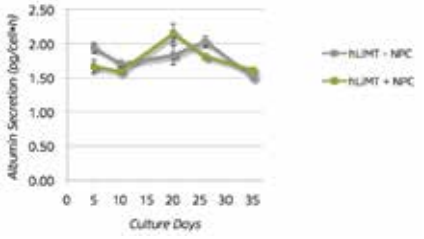
3D InSight™ Liver Microtissues are validated for viability and functionality over 5 weeks in culture.

Day 7 Day 15 Day 19 Day 33

Viability

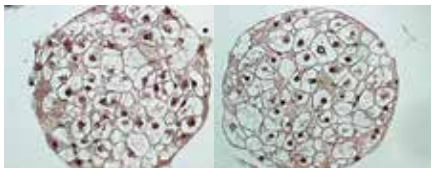


Albumin

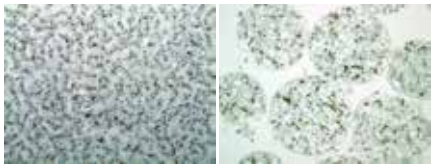


Complex Organotypic Cytoarchitecture

Microtissues consist of polarized hepatocytes with tight cell-cell junctions (H&E staining, top), and an intact bile canaliculi network expressing bile salt export pump (BSEP) protein reflecting that of native liver (bottom left/right).



Day 7 Day 28

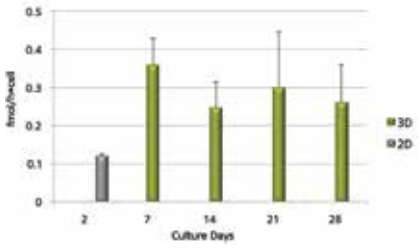


Native Liver Human Liver Spheroids (Day 10)

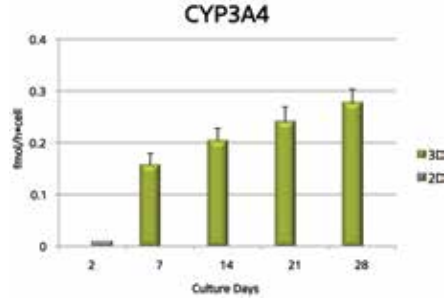
Superior Long-lived Metabolic Activity

Microtissues display increased metabolic enzyme activity compared to 2D culture persisting over a 4 week incubation period.

CYP1A2



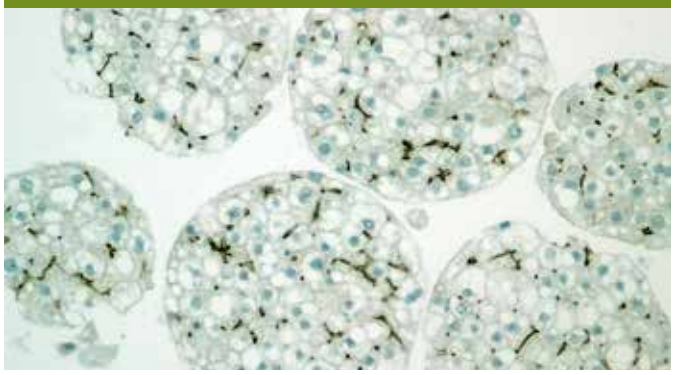
CYP3A4



Advanced Toxicity Assessment in 3D

InSphero 3D culture technology helps you bring far greater assurance to compound de-risking with 3D InSight™ Liver Microtissues and Toxicology Services.

For Your In-house Evaluation



Assay-ready 3D InSight™ Liver Microtissues (p14)

- Fixed production schedule for routine experimental timelines
- Reproducibly manufactured and QC'd to ensure continuity
- Conveniently delivered next-day, ready for dosing
- Multiple donor lots available to meet specific profile needs

Contract Research

Tailored 3D InSight™ Toxicology Services (p18)

- Identify disconnect between existing *in vitro* and *in vivo* toxicity data
- Investigate mechanistic toxicities on clinically troublesome compounds
- Rapidly generate supportive data to justify adoption of 3D platform
- Screen for hepatotoxic compounds at an early stage in development

ENDPOINT ASSESSMENT

Cytotoxicity/ Hepatotoxicity
- » ATP content
- » LDH
- » AST/ALT
- » α-GST
- » Albumin secretion
- » Urea secretion

Kupffer Cell Function
- » IL-6 secretion
- » TNFα secretion

Mitochondrial Impairment
- » Oxygen consumption rate & extracellular acidification
- » Mitochondrial membrane potential

Reactive Metabolite Formation
- » Intracellular GSH content
- » Malondialdehyde accumulation (IHC)

Metabolic Competence
- » CYP activity

Apoptosis
- » Caspase-3/7 activation

Steatosis/ Phospholipidosis
- » Intracellular neutral lipid & phospholipid accumulation

Cholestasis
- » Transporter protein expression
- » Bile acid secretion

Proliferation
- » DNA synthesis

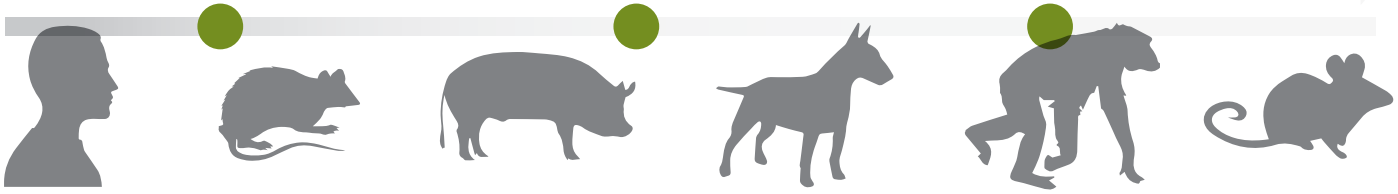
mRNA & Protein Expression
- » Transcriptomics
- » Proteomics

The InSphero Liver Microtissue Pipeline

CURRENTLY AVAILABLE
- Human
- Rat
- HepG2

BETA TEST NOW
- Minipig
- Dog
- HepaRG

LAUNCH Q3/4 2014
- 384-well microtissues
- Cynomolgus monkey
- Mouse



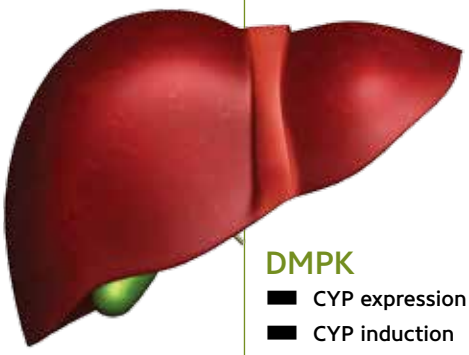
Multiparametric Analysis for ADME-Tox & DMPK

No single assay will tell the whole story when it comes to predicting toxicity. The long-lived, organotypic nature of InSphero 3D InSight™ Liver Microtissues facilitates a multiparametric approach to characterize toxicity and DMPK that are limited or unobtainable using conventional 2D culture.

- **Evaluate repeat dose and long-term, low-dose exposure in a high-throughput *in vitro* format**
- **Use commercially available cell-based assays & immunoassays for mechanism of toxicity analysis**

Mechanistic Toxicity

- Long-term & chronic toxicity
- Idiosyncratic toxicity
- ROS-mediated toxicity
- Apoptosis/Necrosis
- Bile acid transport
- Global proteomic analysis



DMPK

- CYP expression
- CYP induction
- Low clearance compounds

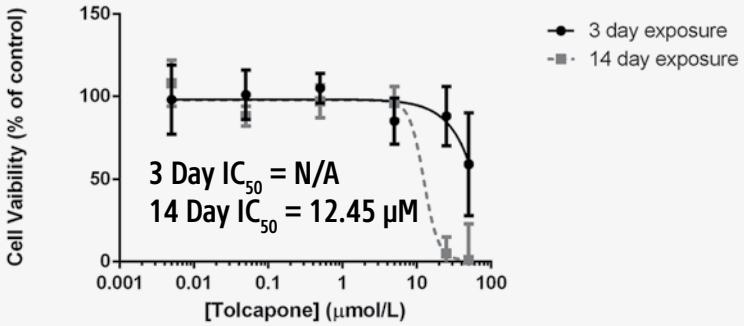
Proof of Principle

Long-term & Chronic Toxicity

Viability Endpoint Assays: ATP, LDH, AST/ALT, α-GST, Albumin, Urea

Principle:
To mimic *in vivo* hepatotoxic effects *in vitro,* a stable, long-lived liver model is required that can be repeatedly dosed over prolonged periods to reveal subtle, sub-acute toxic effects.

Proof:
The ATP content of 3D InSight™ Human Liver Microtissues after 3 day or 14 day treatment with Tolcapone reflects the chronic toxicity profile observed with the drug *in vivo*. ATP content determined using Promega CellTiter-Glo®. Long-term medium exchange/ repeat dosing was performed every 48 hours for 14 days.



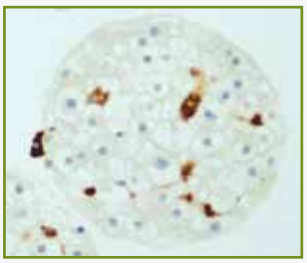
Idiosyncratic Toxicity

Kupffer Function Assays: ATP Assay, IL-6 & TNF-α ELISAs

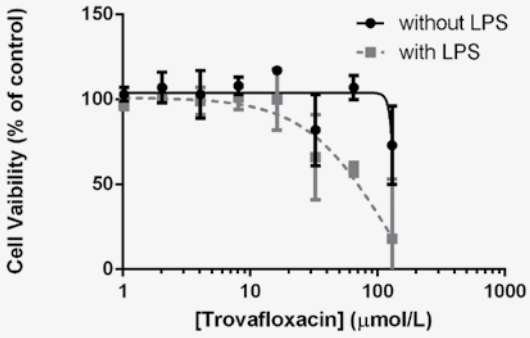
Principle:

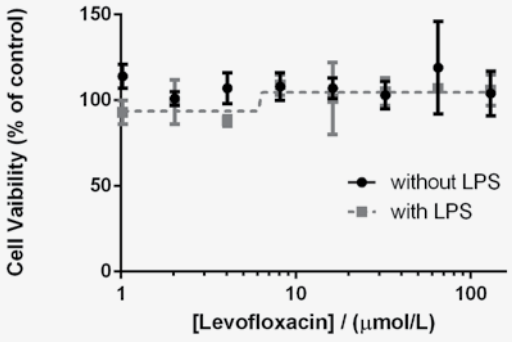
To detect idiosyncratic toxicities, an *in vitro* system must include both hepatocytes and non-parenchymal Kupffer cells capable of simulating a pro-inflammatory environment.

Proof:



InSphero's 3D culture technology allows co-culture of hepatocytes with IL-6 and TNF-α secreting CD68+ Kupffer cells (brown stained cells). Co-exposure of Human Liver Microtissues with Trovafloxacin in the presence of an inflammagen (LPS) reveals toxicity (top graph) not seen with Levofloxacin (bottom).





ROS-mediated Toxicity

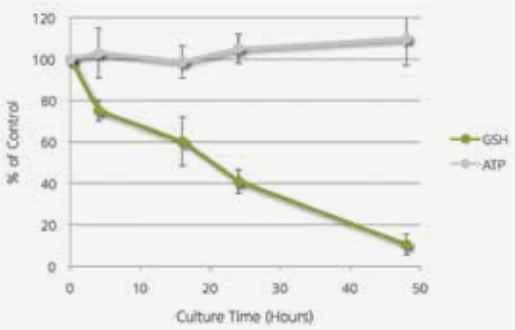
REDOX Endpoint Assays: GSH Content, Oxygen Consumption/Extracellular Acidification

Principle:

A useful liver model should allow monitoring of cellular redox status to reveal excess reactive oxygen species (ROS) or glutathione (GSH) depletion, such as GSH depletion induced by the reactive Acetaminophen metabolite NAPQI.

Proof:

Treatment with buthionine-sulfoximine (BSO), a GSH-synthesis inhibitor, can be used to mimic decreased GSH levels in liver microtissues, as measured by GSH-Glo™ (Promega). BSO treatment depletes GSH levels (green line) while maintaining cell viability, as measured by ATP content (gray line).

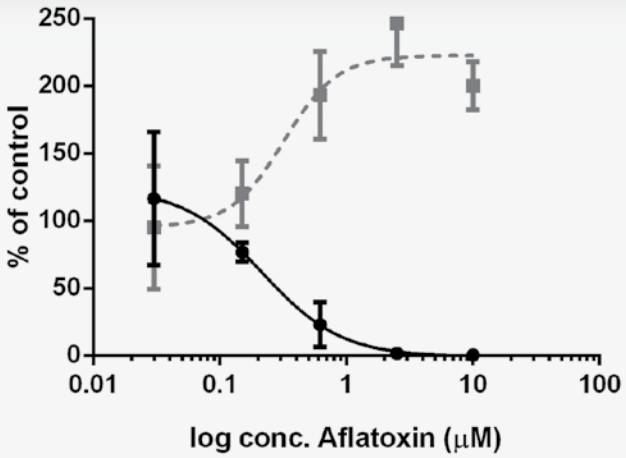


Apoptosis/Necrosis

Cell Death Assays: Caspase 3/7 activity, ATP

Principle:
To pinpoint triggering of programmed cell death in response to toxic insult, an in vitro model should display responsiveness of intrinsic apoptotic pathways (e.g. Caspase 3/7 activation).

Proof:
Apoptotic cell death is observed in a dose-dependent manner following treatment of Human Liver Microtissues with Aflatoxin. The amount of cleaved, activated executioner caspases can be measured using Promega's Caspase-Glo® 3/7 Assay (gray dashed line), and is mirrored by a corresponding decrease in viability (ATP – black solid line).

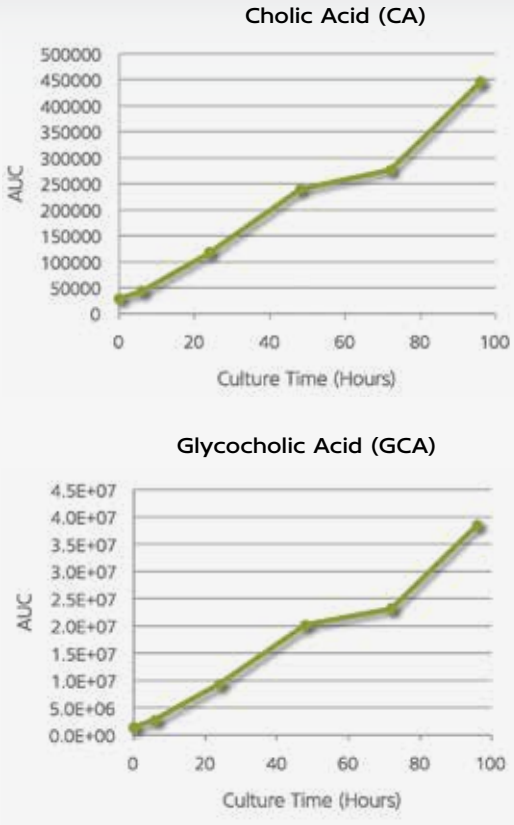


Bile Acid Transport

Bile Acid Profile Analysis: LC-MS Profiling of Microtissue Supernatants

Principle:
To model altered bile acid profiles seen in cholestasis, a liver model must contain the cytoarchitecture and specialized transporters (i.e. BSEP & MRP2) to support bile acid conjugation and secretion into the bile canaliculi and media.

Proof:
The intact bile-canalicular network in 3D InSight™ Human Liver Microtissues make them an ideal tool to study effects of transporter inhibition. High-sensitivity LC-MS enables profiling of up to 13 different bile acids, including cholic acid (top) and Glycocholic acid (bottom), in microtissue supernatants.



Bile acid transport analysis performed in collaboration with Pharmacelsus GmbH

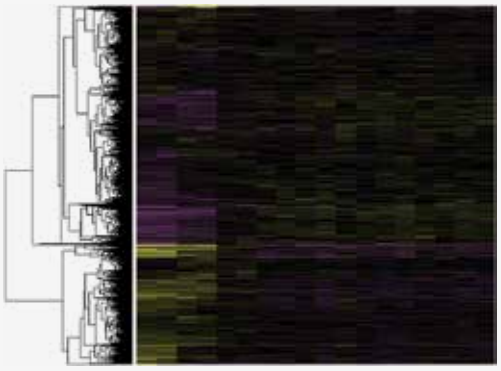
Global Proteomic Analysis

Novel Pathway & MOA Identification: HRM Mass Spectroscopy

Principle:
A robust microtissue model should facilitate global proteomic analysis, providing an unbiased view of mode of action (MOA) and mechanism of toxicity (MOT).

Proof:
Significant changes in global protein expression are observed at sub-toxic doses of acetaminophen in Human Liver Microtissues using state-of-the-art mass spec to monitor >2400 proteins. Unbiased clustering of protein profiles (heatmap, right), combined with functional annotation clustering, identify upregulation of CYP1A2, CYP2E1 and CYP3A4 enzymes, along with changes in more than 160 proteins. Importantly, downregulation of icosanoid metabolism and inflammation, both known acetaminophen MOAs, are detected.

Global Proteomics Identifies Regulatory Pathways & Drug MOA



Proteomic analysis performed in collaboration with Biognosys AG

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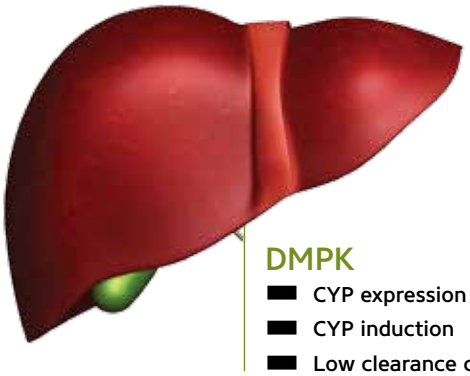
- ✔ Latest 3D News
- ✔ Webinars/Conferences
- ✔ User Group Meetings
- ✔ And more...

An Improved Organotypic Model for *In Vitro* DMPK Analysis

Why is 3D > 2D for DMPK?		
	2D Hepatocyte Monolayers	3D Liver Microtissues
Viability	Days	4+ weeks
CYP Expression	De-differentiation starting after 8h in 2D	Broad & Persistent
Uptake/ Secretion	Transporters expressed	Transporters & intact bile canaliculi network
Cells Required	$1x10^5 - 4x10^5$ /well	$1x10^3$/MT



DMPK
- CYP expression
- CYP induction
- Low clearance compounds

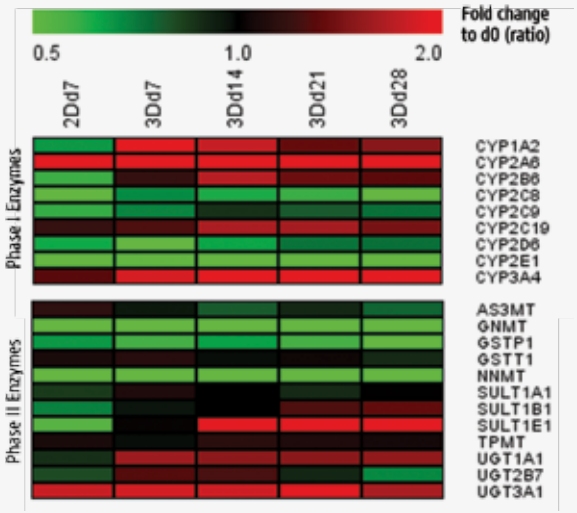
Proof of Principle

CYP Expression

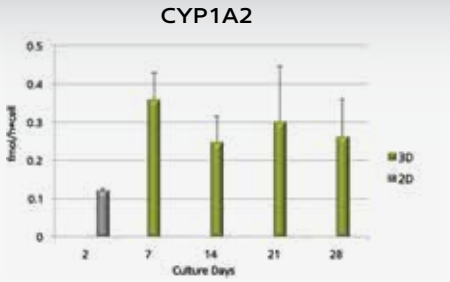
CYP Expression & Activity Assays: Whole Genome Array, LC-MS metabolite detection

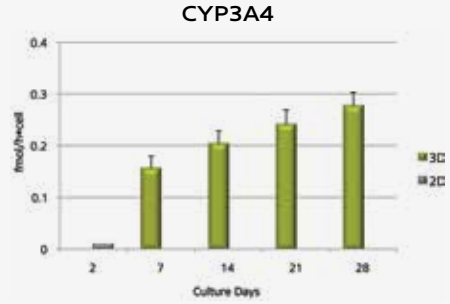
Principle:
To determine if a drug is a substrate, inhibitor, or inducer of metabolizing enzymes, an *in vitro* model should include robust, stable, and inducible CYP expression that mimics *in vivo* metabolism. Such profiling can help predict drug-drug interactions.

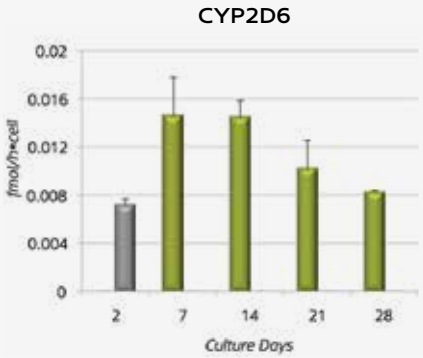
Proof:
3D Human Liver Microtissues display increased, persistent Phase I & II enzyme mRNA levels compared to 2D (heat map below, red shading indicates ~2-fold change at time point relative to day 0). Likewise, microtissues were incubated with CYP substrates, and metabolites/products from single microtissues were detected in supernatants by LC-MS. 3D cultures displayed higher basal levels of CYP1A2, CYP3A4, and CYP2D6 activity compared to 2D, and activity persisted up to 28 days in culture.



CYP Expression performed in collaboration with Karolinska Institute and Biognosys AG





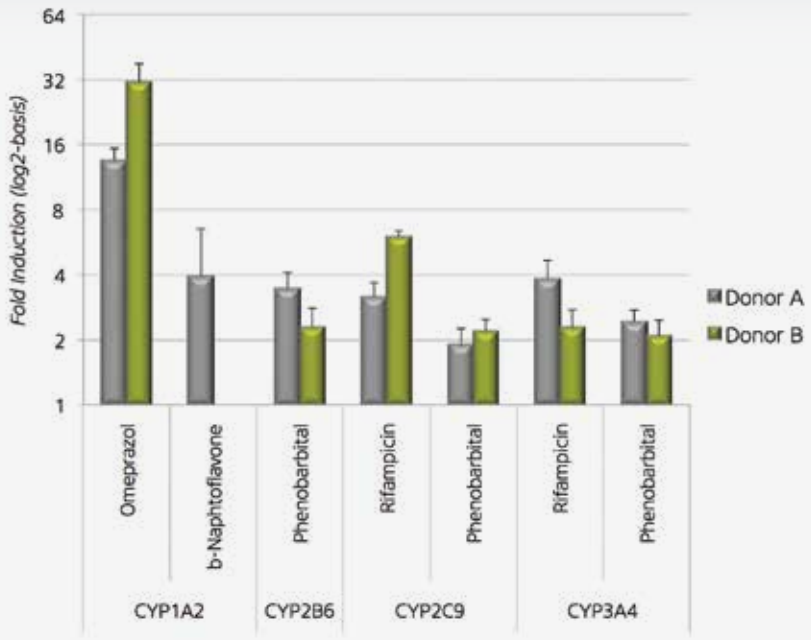


CYP Induction

Metabolic Enzyme Induction: LC-MS metabolite detection

Principle:
In addition to displaying CYP enzyme expression, an in vitro model should demonstrate CYP inducibility that mimics in vivo metabolic responsiveness to known pharmacological modulators.

Proof:
Responsiveness of CYPs to induction is a critical parameter for predicting potential drug-drug interactions. Human Liver Microtissues from two individual donors co-cultured with Kupffer cells were exposed to prototypic CYP-inducers for 72h with daily medium exchange. For the detection of CYP1A2, 2B6, 2C9 and 3A4 activity, a substrate cocktail was added for 24h to control tissues and CYP-induced samples, before analysis of corresponding metabolites. The fold induction above control tissues is depicted.



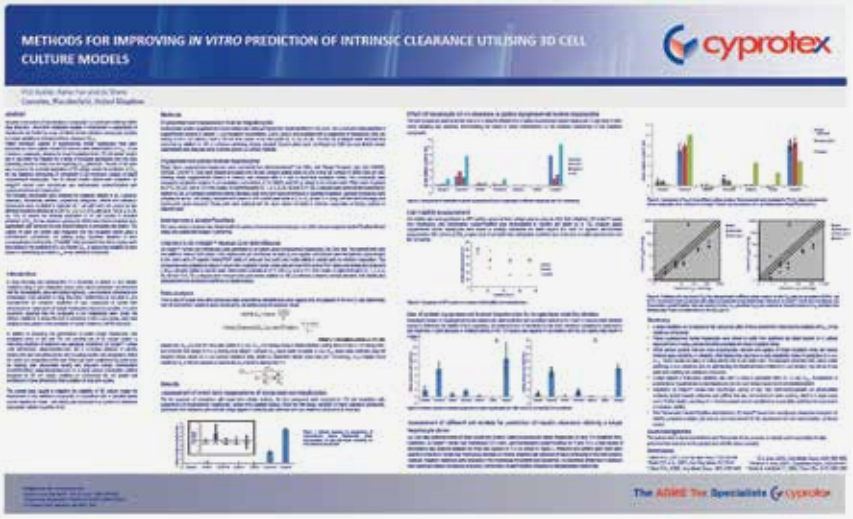
CYP induction analysis performed in collaboration with Pharmacelsus GmbH

Low Clearance Compounds

Intrinsic Clearance Assays: LC-MS analysis of supernatants

Principle:
To assist in determining bioavailability, half-life, and dosing regimen during discovery and development, an *in vitro* model with an organotypic metabolic profile can be a powerful tool to predict *in vivo* hepatic clearance.

Proof:
Download the Cyprotex poster from ISSX 2013 at www.insphero.com to learn more about the ability of 3D InSight™ Human Liver Microtissues to predict intrinsic clearance of low clearance compounds.



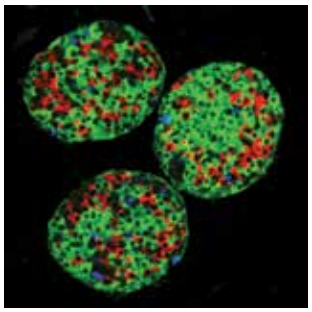
Find this poster and more at www.insphero.com

Beyond 3D Liver Models:
Emerging 3D microtissue models for toxicity testing

Toxicities observed in the heart, pancreas and brain are additional sources of drug attrition, and thus of concern during the development process. The InSphero pipeline includes a panel of organotypic microtissues for investigating potential toxic liabilities in non-liver organs.

3D InSight™ Human & Rat Pancreatic Microislets

- **Viability and glucose-stimulated insulin secretion persist > 4 weeks, enabling long-term studies**
- **Homogeneous microislet size eliminates tedious hand-picking**
- **Uniform ratio of alpha, beta, and delta cells minimizes islet-to-islet variation**



Immunofluorescent staining of Human Microislet cross-sections displaying expression of insulin (green, beta cells), glucagon (red, alpha cells), and somatostatin (blue, delta cells) in various islet cell sub-types.

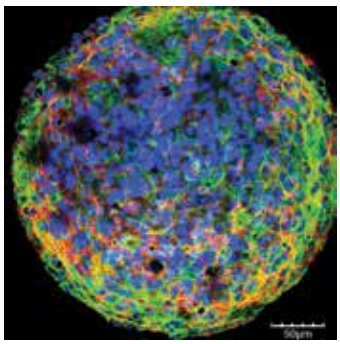
3D InSight™ Human & Rat Myocardial Microtissues

- **Established from iPS-derived human or neonatal rat cardiomyocytes**
- **Multi-cell type cardiac microtissues display viability and contractility for >3 weeks**
- **Demonstrate temperature- and pharmacologically-sensitive contractile responsiveness**

View neonatal rat cardiomyocyte 3D microtissues beating synchronously at www.insphero.com

3D InSight™ Rat Neuronal Microtissues

- **Established from newborn rat brain (cortex) isolates**
- **Display stable size and viability for >28 days**
- **Demonstrate organotypic expression of β-III tubulin, Nestin, and GFAP**



Immunofluorescent staining of rat brain microtissues at day 5 of culture for GFAP (green) and β-III tubulin (red). Nuclei stained with DAPI (blue).

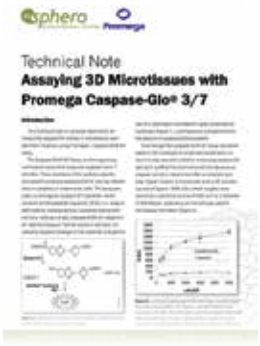
Interested in beta testing?

Contact us for more information about these and other 3D InSight™ microtissue models.

Further Reading

Discover more Applications, Webinars and Technical Notes at www.insphero.com

Multi-parametric measurements of ATP and DNA in Microtissues



Assaying 3D Microtissues with Promega Caspase-Glo® 3/7

General References:

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Kelm J.M. and Lichtenberg, J. (2013). "3D Cell Culture for Compound De-Risking" Innovations in Pharmaceutical Technology, Zurich.

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Human Liver Microtissues:

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Rat Liver Microtissues:

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HepG2 Liver Microtissues:

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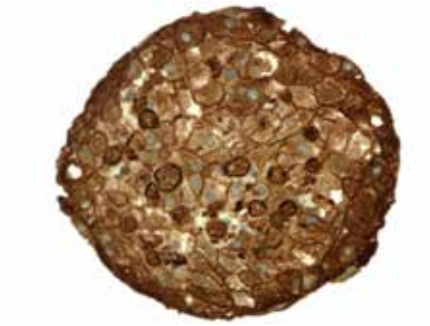
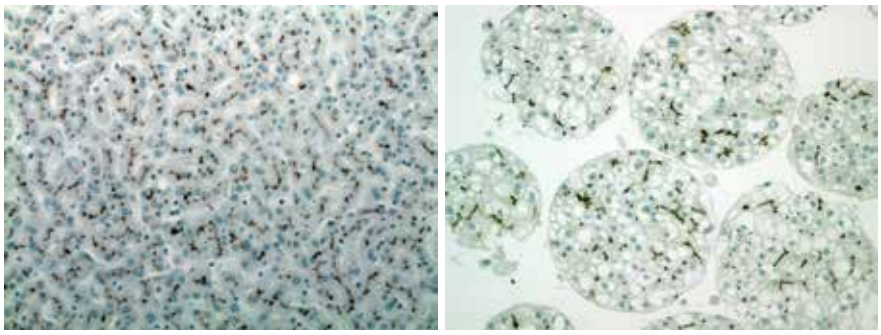
3D InSight™ **Human Liver Microtissues**

96 Assay-ready Microtissues

- Co-culture of primary hepatocytes & NPCs, including Kupffer cells
- Suitable for idiosyncratic and long-term toxicity with repeated dosing
- Economical and convenient 96-well format
- Delivered next-day

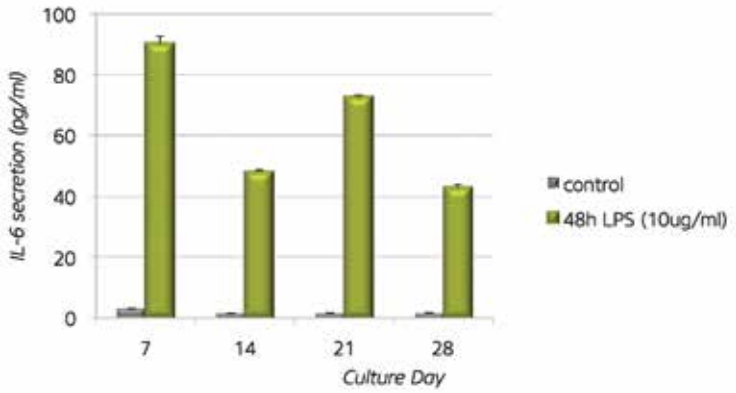


About this image:
Cytokeratin 8 antibody staining by IHC.



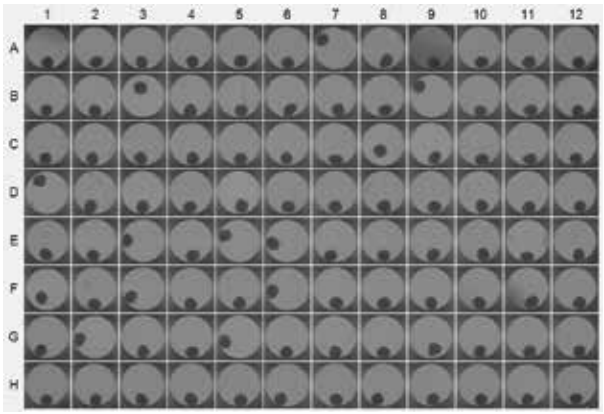
Native Human Liver | 3D InSight™ Human Liver Microtissues (day 10)

Organotypic cytoarchitecture
The intact bile canaliculi network (BSEP expression, brown staining) mirrors that seen in native human liver.



Hepatocyte/Kupffer cell co-culture facilitates idiosyncratic toxicity testing

IL-6 secretion in control (gray) or LPS-stimulated (green) Human Liver Microtissue supernatants, assessed by ELISA.



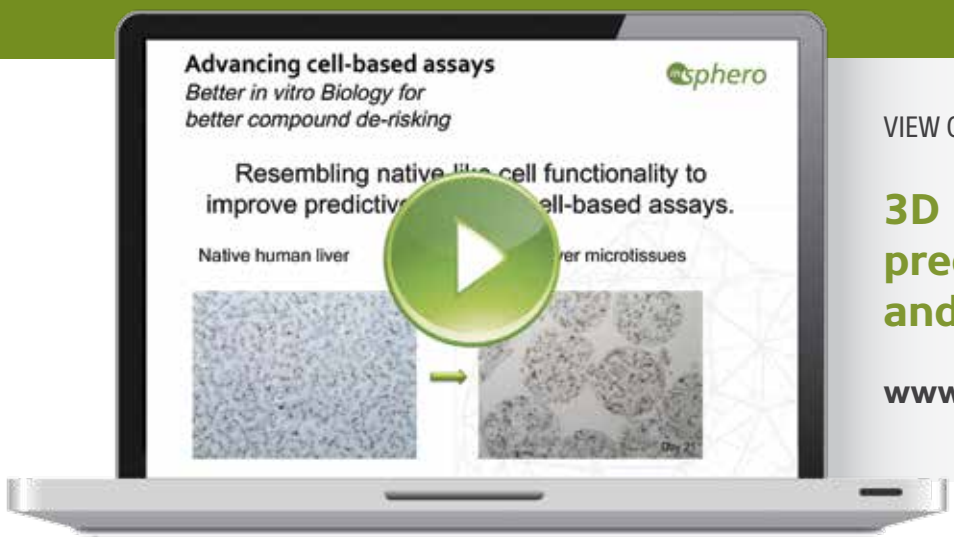
Ensure reproducibility with <5-7% well-to-well variability in microtissue diameter

Image capture of 96-wells of 3D InSight™ Human Liver Microtissues in a single GravityTRAP™ plate.

Product Specifications	
Delivery format	GravityTRAP™
Microtissues per plate	96, one microtissue per well
Inoculation cell number	1000 hepatocytes per microtissue; optional: NPCs
Cell source	Cryopreserved primary cells
Microtissue re-aggregation time	4 days
Time in culture upon delivery	6 days
Microtissue size upon delivery	220-320 µm
Size distribution (rel.SD)	<5-7% of mean
ATP content upon delivery	>10 pmol/MT
Maintenance medium	CS-07-001; 3D InSight™ Human Liver Maintenance Medium, serum-free (500mL)
Medium exchanges	2-3 times a week
Cultivation volume	50-70 µl
Culture life-time	>4 weeks

Catalog #	Product
MT-02-002-01	3D InSight™ Human Liver Microtissues from primary hepatocytes (96x)
MT-02-002-04	3D InSight™ Human Liver Microtissues from primary hepatocytes, co-culture with non-parenchymal cells (96x)
CS-07-001	3D InSight™ Human Liver Maintenance Medium, serum-free (500mL)

Want to Learn More?



VIEW OUR WEBINAR:

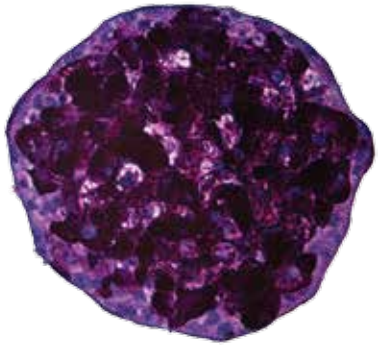
3D liver models for more predictive toxicology and drug safety testing

www.insphero.com

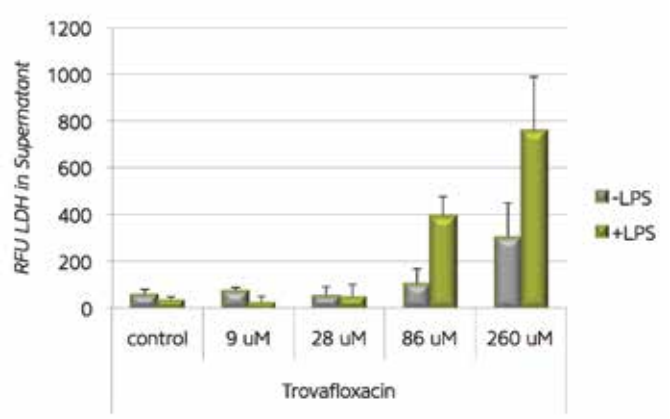
3D InSight™ **Rat Liver Microtissues**

Assay-ready in vitro rat model system

- Co-culture of fresh primary hepatocytes & Kupffer cells (NPCs)
- Suitable for idiosyncratic and chronic toxicity with repeated dosing
- Economical and convenient 96-well format
- Delivered next-day



About this image:
PAS staining of Rat Liver Microtissue confirms glycogen storage.



Monitor Inflammation-mediated toxicity:
Co-exposure of rat liver microtissues with Trovafloxacin and an inflammagen (LPS) is identified as toxic, as evidenced by leakage of LDH into microtissue supernatants.

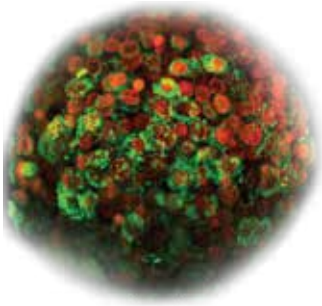
Product Specifications	
Delivery format	GravityTRAP™
Microtissues per plate	96, one microtissue per well
Inoculation cell number	1000 hepatocytes per microtissue; optional: NPCs
Cell source	Fresh
Microtissue re-aggregation time	4 days
Time in culture upon delivery	6 days
Microtissue size upon delivery	220-320 µm
Size distribution (rel.SD)	<5-7% of mean
ATP content upon delivery	>5 pmol/MT
Maintenance medium	CS-07-002; 3D InSight™ Rat Liver Maintenance Medium, serum-free (500 mL)
Medium exchanges	2-3 times a week
Cultivation volume	50-70 µl
Culture life-time	>3-4 weeks

Catalog #	Product
MT-02-001-01	3D InSight™ Rat Liver Microtissues from primary hepatocytes (96x)
MT-02-001-04	3D InSight™ Rat Liver Microtissues from primary hepatocytes, co-culture with nonparenchymal cells (96x)
CS-07-002	3D InSight Rat Liver Maintenance Medium, serum-free (500 mL)

3D InSight™ **HepG2 Liver Microtissues**

Your easiest option for early-stage hepatotoxicity screens

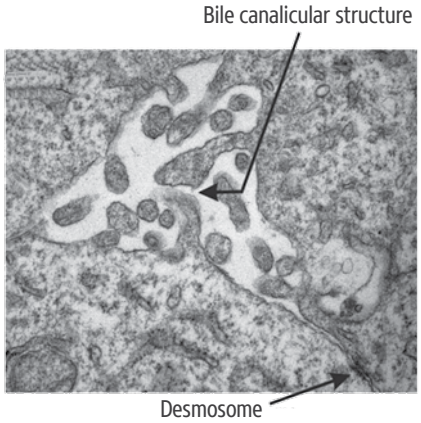
- Compare previous 2D HepG2 test results to 3D data
- More organotypic functionality in 3D culture
- Long-term (>10 days) studies of drug effects possible
- Economical and convenient 96-well 3D format
- Delivered next-day



About this image:
Light-sheet microscopy (mDSLM) image of HepG2 Microtissue. Maximum projection of 2000 cells based on 310 stacks of 1µm. DRAQ5™ nuclear staining (red) and MitoView™ Green mitochondria staining (green). Image provided gratefully by Nariman Ansari, Francesco Pampaloni and Ernst H. Stelzer, Goethe University Frankfurt.

Improved Characteristics of 3D HepG2

- » Cuboidal cell shape
- » Formation of bile canaliculi
- » ↑ albumin and glutamate production
- » ↑ CYP450 induction
- » ↑ drug efflux activity



HepG2 microtissue cytoarchitecture
Microtissue section, displaying presence of bile canaliculi and tight cell-cell contacts with desmosomes. From J.M Kelm et al. (2003) Biotechnology and Bioengineering.

Product Specifications	
Delivery format	GravityTRAP™
Microtissues per plate	96, one microtissue per well
Inoculation cell number	200 cells per microtissue; optional: NPCs
Cell source	ATCC, cryopreserved
Microtissue re-aggregation time	4 days
Time in culture upon delivery	6 days
Microtissue size upon delivery	150-250 µm
Size distribution (rel.SD)	<10% of mean
ATP content upon delivery	>5 pmol/MT
Maintenance medium	CS-07-101, 3D InSight™ Cell Line Maintenance Medium (500mL)
Medium exchanges	2-3 times a week
Cultivation volume	50-70 µl
Culture life-time	>2-3 weeks

Catalog #	Product
MT-02-100-01	3D InSight™ HepG2 Liver Microtissues (96x)
CS-07-100	3D InSight™ Cell Line Maintenance Medium (500mL)

3D InSight™ **Toxicology Services**

The world's largest 3D-focused contract research organization

- ✔ Tailored screening conducted and interpreted by our staff of 3D microtissue experts
- ✔ Enable long-term testing for low-dose & repeat-dose experiments
- ✔ Choose from a varied menu of endpoint assays offered in-house, or through our network of partners
- ✔ Services fully customizable to meet your requirements
- ✔ Rapid turnaround with strict confidentiality

1 Choose Your Compounds

2 Select Your 3D Model
(Human, Rat, HepG2, Dog, Minipig)

3 Choose Your Endpoints

Explore Multiple Toxicity Mechanisms

4 We Screen
(Multiple dilutions, Repeat dosings)

5 We Deliver
(Web-based presentation, Discussion with our 3D experts)

Visit us at www.insphero.com/services or contact your local branch office or authorized distributor to discuss a plan that suits your needs.

3D Compatible Endpoints

A variety of amenable assays

Toxicity Mechanism	Cellular Indicator	3D-Compatible Endpoint Assay
Cytotoxicity/Hepatotoxicity	ATP-content LDH (released/intracellular) AST/ALT (released/intracellular) α-GST (released/intracellular) Albumin secretion Urea secretion	CellTiter-Glo® (Promega) CytoTox-ONE™ (Promega) Fluitest® AST/ALT (Analytikon) α-GST-ELISA (EKF-Diagnostics) Albumin-ELISA (Bethyl Laboratories) Urea-Assay (Biovision)
Kupffer-cell function	IL-6 secretion TNF-α ELISA	IL-6 ELISA (Life Technologies) TNF-α ELISA (Life Technologies)
Mitochondrial impairment	Oxygen consumption rate/ extracellular acidification Mitochondrial membrane potential	XF-Flux (Seahorse Biosciences) HCA with TMRM-dye (Life Technologies)
Reactive metabolites	Intracellular GSH content Malondialdehyde generation	GSH-Glo® (Promega) IHC for MDA (Lipid-peroxidation)
Metabolic competence	Cytochrome activation/ induction	P450-Glo™ (Promega) Enzyme activity (Pharmacelsus)
Apoptosis	Caspase 3/7 activation	Caspase-Glo® 3/7 (Promega) HCA with CellEvent (Life Technologies)
Steatosis, phospholipidosis	Lipid accumulation	HCS-LipidTOX™ for Steatosis and Phospholipidosis (Life Technologies) BODIPY (Life Technologies)
Cholestasis	Transporter proteins Bile-acid secretion	IHC for BSEP LC-MS analysis (Pharmacelsus)
Proliferation	DNA-synthesis	EdU Click-iT® (Life Technologies)
mRNA & Protein Expression Analysis	Transcriptomics Proteomics	PCR-Arrays (SA-Biosciences); Affymetrix® Arrays HRM-MS (Biognosys)

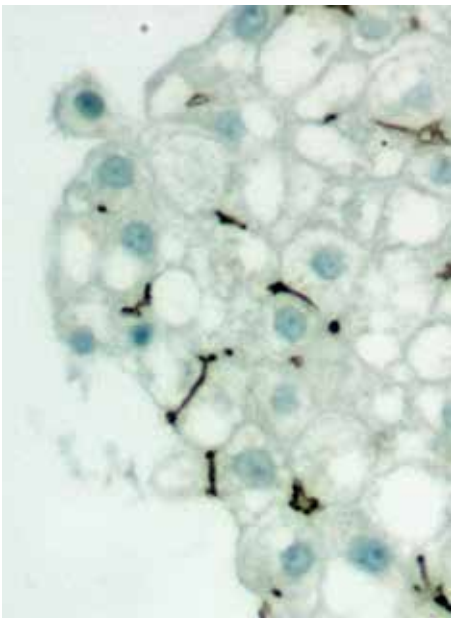
3D InSight™ Liver Toxicology Service Packs

Screening simplified

If a custom multi-endpoint project is beyond your needs, our 3D InSight™ Liver Toxicology Service Packs are designed to simplify the service process. Just send us your compounds, choose your 3D model, and we deliver a report in 2-4 weeks for one convenient price. Each pack includes toxicity assessment by ATP content for 5 to 20 compounds after 14 days of treatment.

Catalog#	Product
SP-02-001-00	3D InSight™ Liver Toxicology Service Pack – 5 Compounds
SP-02-002-00	3D InSight™ Liver Toxicology Service Pack – 10 Compounds
SP-02-003-00	3D InSight™ Liver Toxicology Service Pack – 20 Compounds

inSphero
Biomimetic Microtissue Technology



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