A 3-D Biomimetic Liver Platform for Predicting Toxicity in Humans

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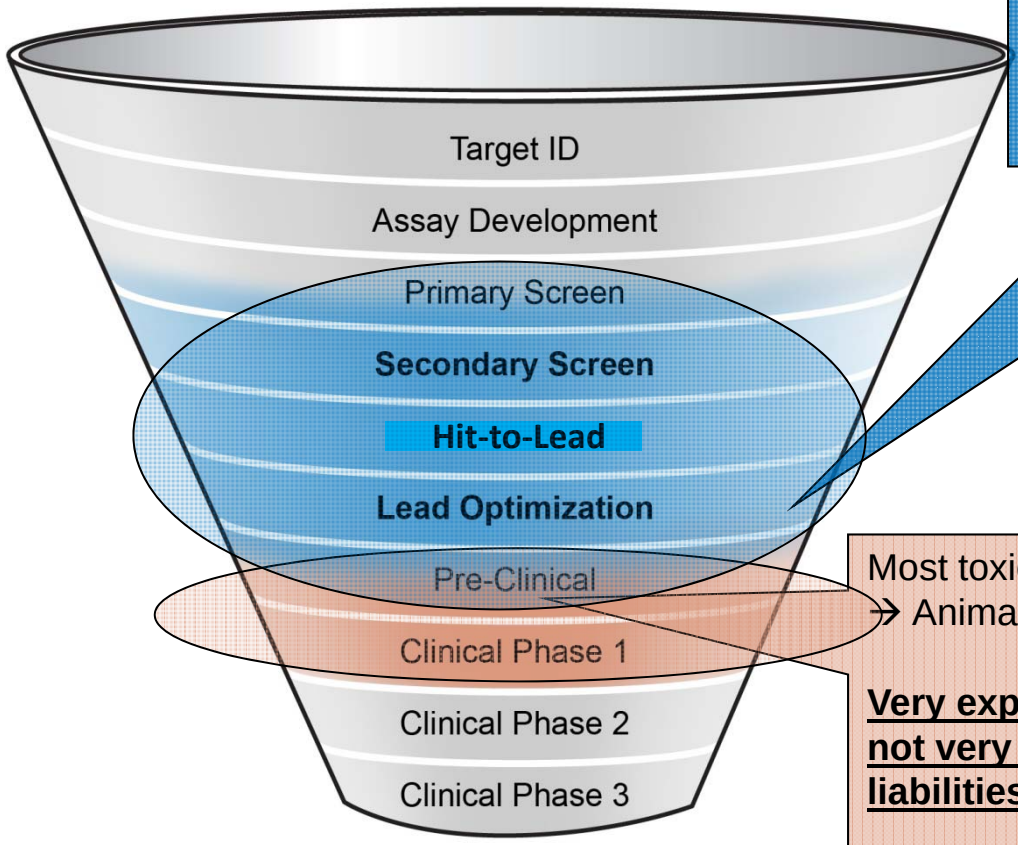
Why are Human 3D Tissue Models Needed?



EARLY SAFETY ASSESSMENT is the process of implementing **faster, less expensive, and more predictive** methods to identify toxic liabilities EARLY in the drug discovery process.

Most toxicology is performed in Pre-Clinical Phase → Animal Studies → Phase I Clinical Trials.

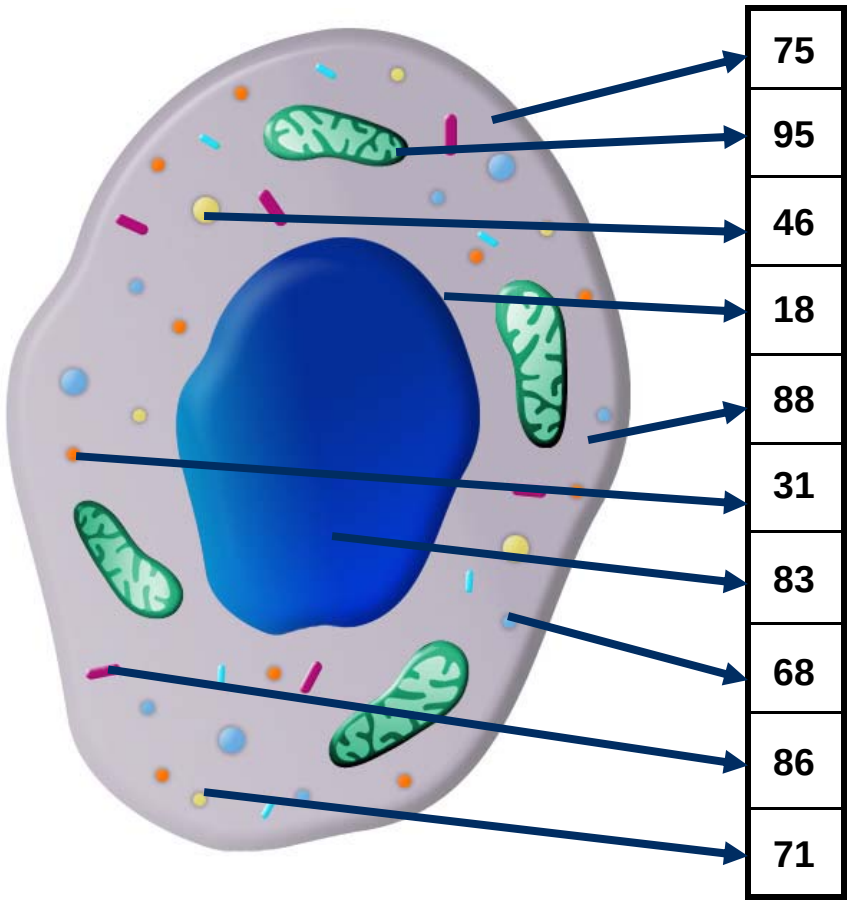
Very expensive, time consuming, animal models not very predictive of many human toxic liabilities, exhibits high failure rate

NRC, *Toxicity Testing in the 21st Century: A Vision and a Strategy* (National Academies Press, Washington, DC, 2007).

Cellular Systems Biology Approach

Previous 2D Model from Cellumen, now part of Cyprotec

The cell is an integrated and interacting network of genes, proteins & metabolic processes that gives rise to function



Components of CSB

- Standard HCS Imaging Platforms
- HepG2 and Primary Rat Hepatocytes in 2D
- Reagents, Biomarker Panels & Assay Profiles
- Informatics & Classifiers
- Safety Reference Database

Vernetti, L, W. Irwin, K. A. Giuliano, A. Gough, K. Johnston and D. L. Taylor. 2009. Cellular Systems Biology Applied to Preclinical Safety Testing: A Case Study of CellCiphr Profiling. In/Drug Efficacy, Safety and Biologics Discovery: Emerging Technologies and Tools. (S. Ekins S. Ekins and J. Xu, eds.). John Wiley & Sons, N. J. pp. 53-73.

Cellumen CellCiphr ™ Analysis

Cell Features	Measurement
Cell Loss	Cell Number
DNA Fragmentation	Nuclear texture
Nuclear Size	Nuclear Diameter
Apoptosis	Cytochrome C release
DNA Damage Response	GADD153 expression
Mitochondrial Function	Mitochondrial potential
Phospholipidosis	Lysosome expansion
Steatosis	Neutral lipid accumulation

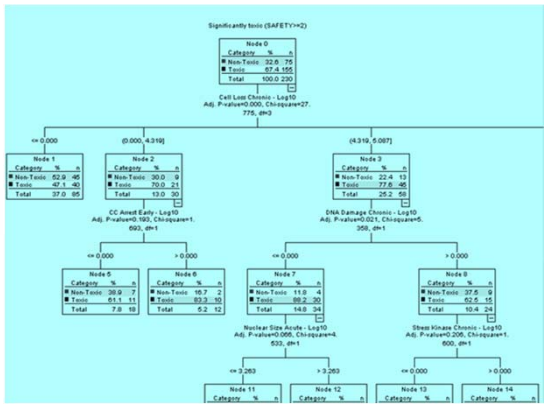
Reference Cpds

Physical Properties

Safety Toxicology

CellCiphr Profiles

Customer Cpds

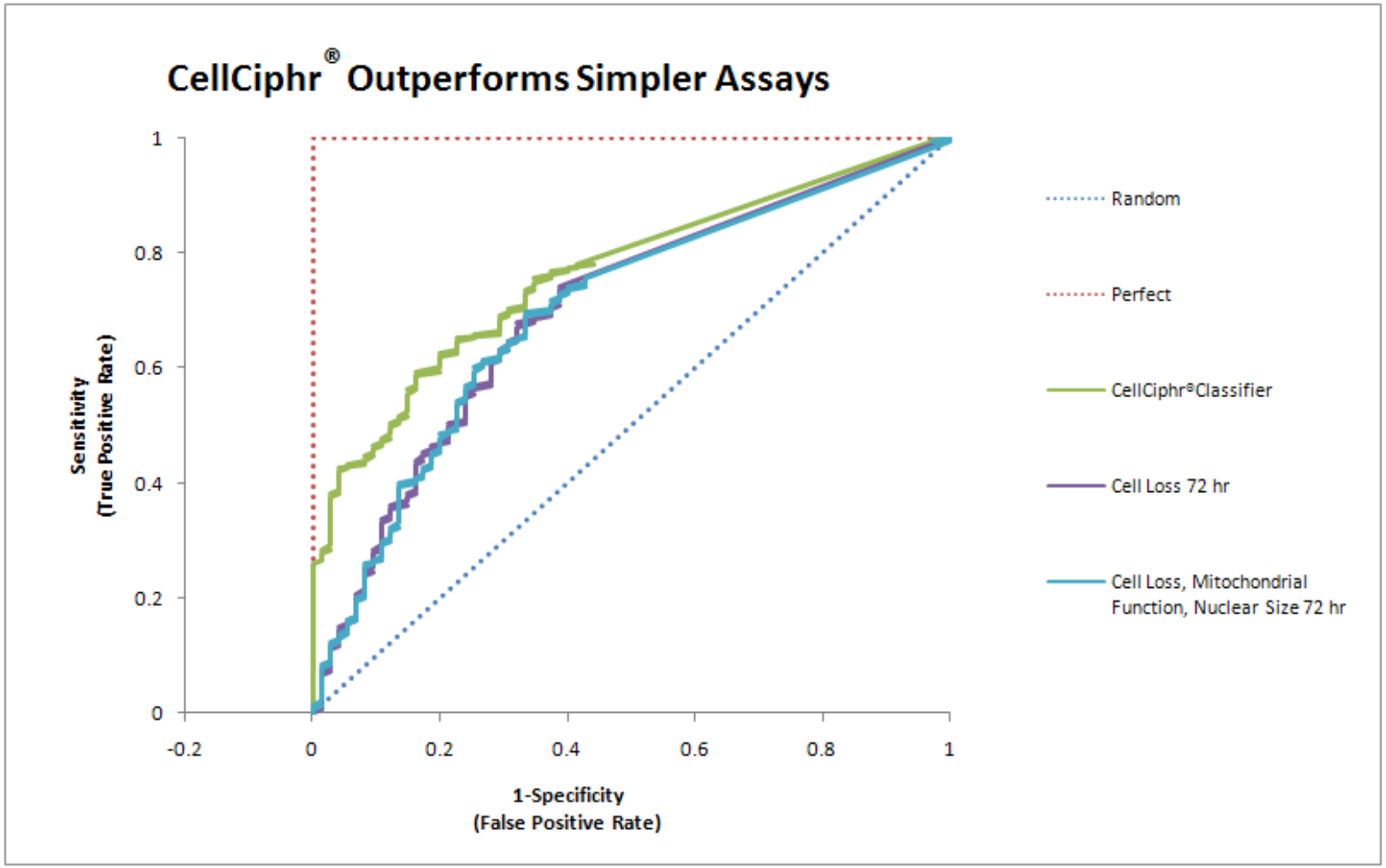


Rank Order

Similarity Profiles

Safety Alert Prediction

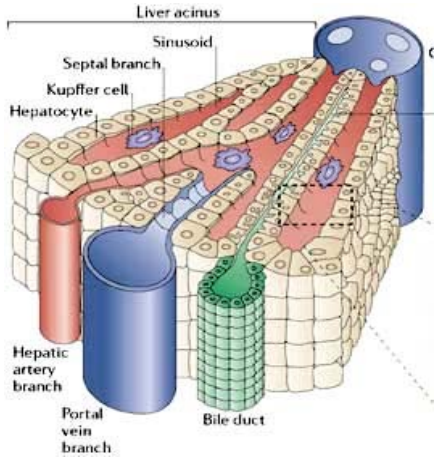
CellCiphr ROC in 2010



Can we do better with Live 3D human models with flow?

Design of the 3D BIOMIMETIC LIVER Device

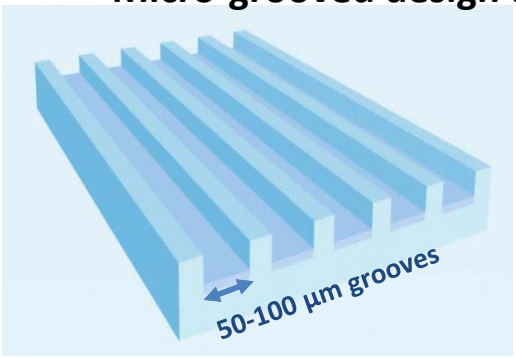
Inspired by the Liver Acinus

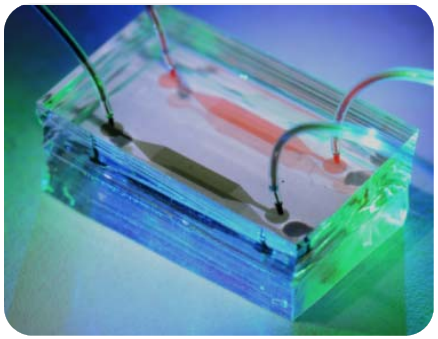


Adams *et al. Nat. Rev.Immunology* **6**, 244–251 (2006)

The Device

Micro-grooved design to create hepatic cords

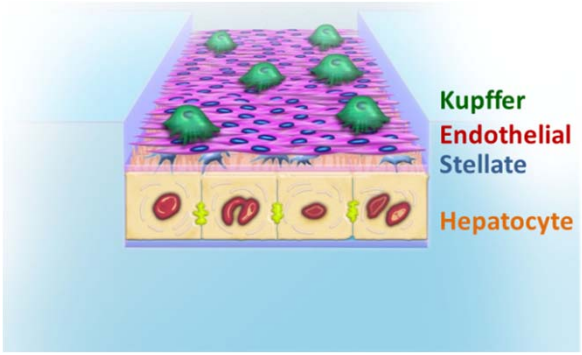


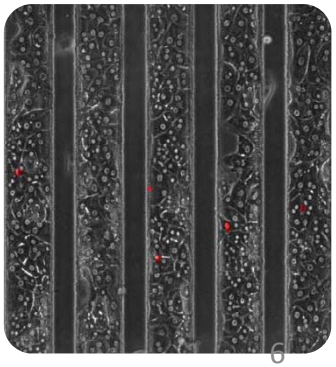


Physiologies to Capture

- **Cellular Mechanisms of Action**
- **Albumin, Urea, LDH leakage, Glucose**
- **Drug metabolism**
- **Zonation (O_2, Chemical)**
- **Bile Production**

Each groove represents a sinusoid with all the essential cell types of the liver





Biomimetic Liver Platform Overview

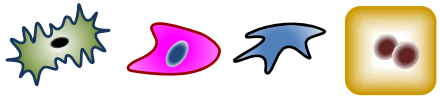
Broad Platform Goals

Reduce Drug Attrition Rates by

- Recapitulating Liver Physiology
- Predictive Database Modeling

Cell Characterization/ Validation Data

Primary Liver Cells



Sentinel Cells

External Data

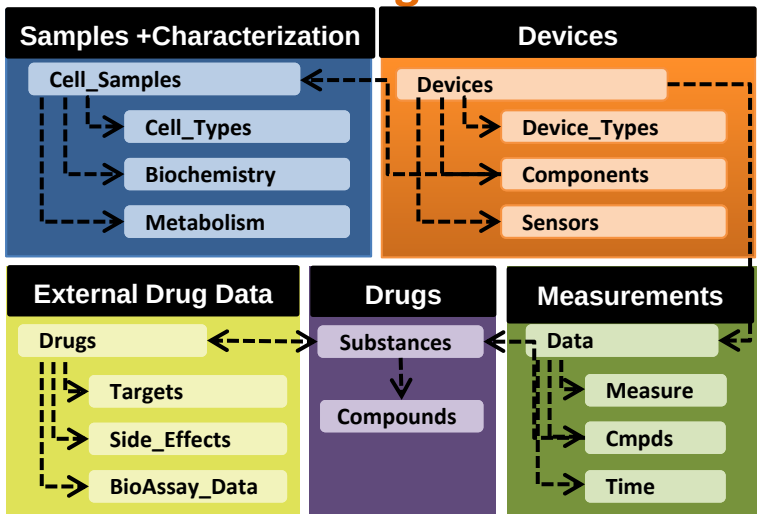
RCSB PDB PROTEIN DATA BANK

ChEMBL

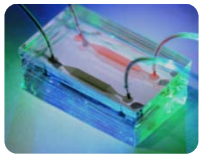
SIDER 2 Side Effect Resource

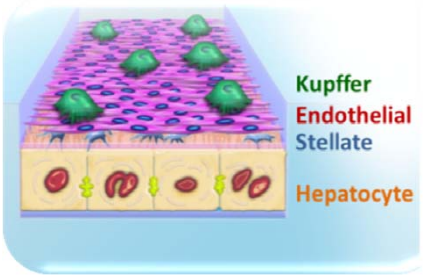
PubChem

Predictive Drug Database



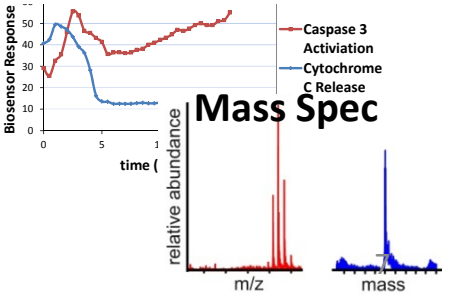
3-D Microfluidic Liver Development





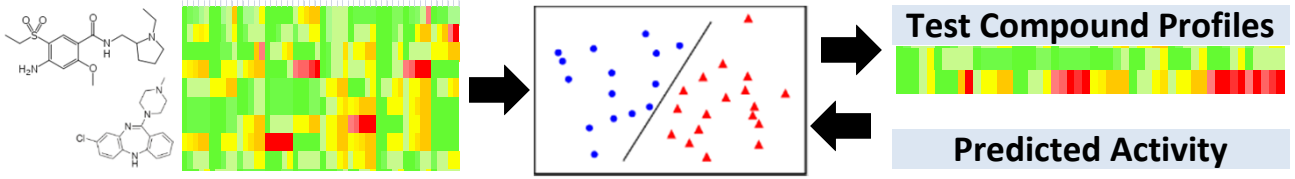
Measurements / Compound Activity

Biosensor/Biochem



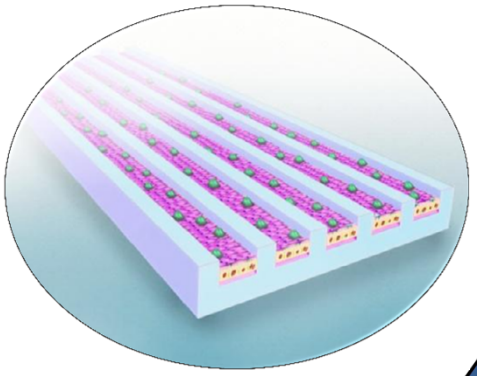
Predictive Model

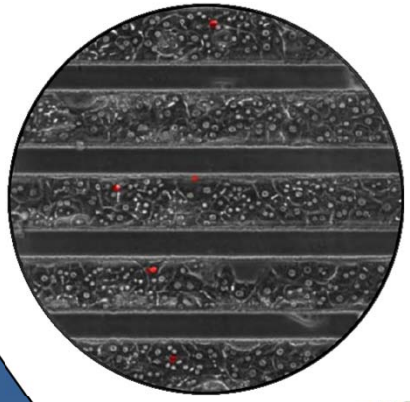
Activity profiles for similar compounds (2D, 3D, target, or bioactivity similarity)

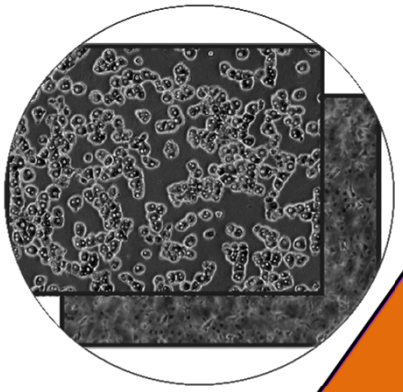


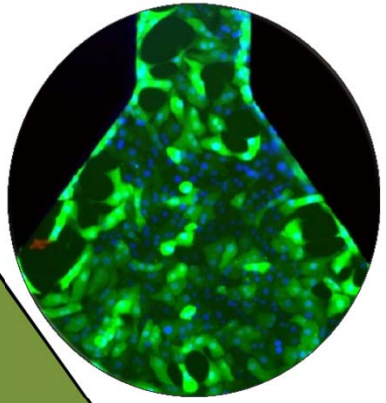
Working Strategy For Building Devices

A Multi-tiered Approach









UH3 Integration

Final UH2 Device: 3D Human Liver Sinusoid

Plate Studies
Initial Testing and Optimization of Culture Conditions

Simplified Device
Sensor and Multi-Cell Integration & Microfluidic Optimizations

In-House Cell Sourcing
Primary Cell Isolation & Human Cell Line and Human IPS Cell Expansion

Biosensors

"Sentinel" Cell Reporters

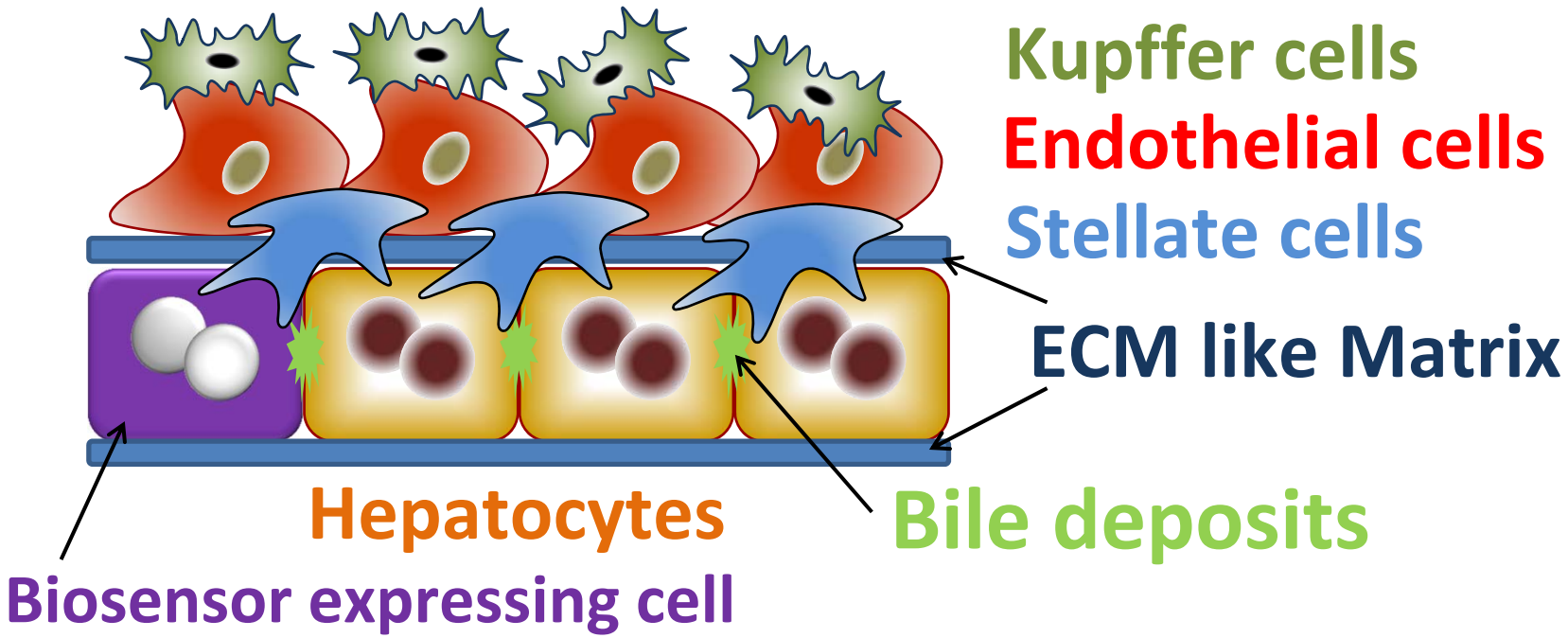


Diagram of sentinel cell approach (above). Our liver acinus model is constructed to contain a subpopulation of hepatocytes, stellate and Kupffer cells that stably express biosensors to monitor distinct cell events. A hepatocyte expressing a biosensor is depicted in purple.

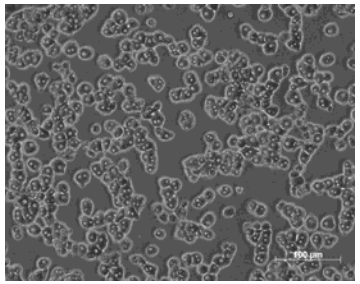
Biomimetic Liver Module - Animation

Results from Plate Cultures

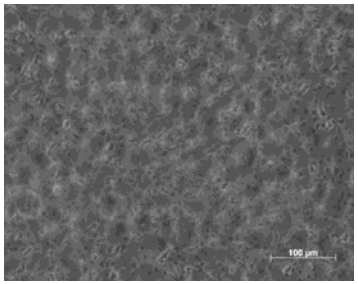
Hepatocyte – LSEC Coculture (2-4 weeks)

Day 2

Hepatocyte Plane

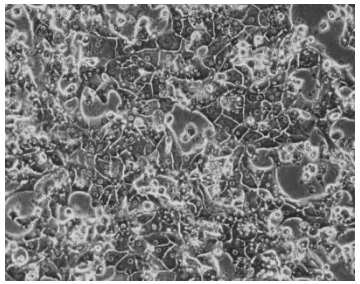


LSEC Plane



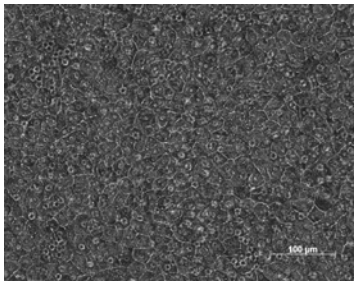
Day 7

Hepatocyte Plane



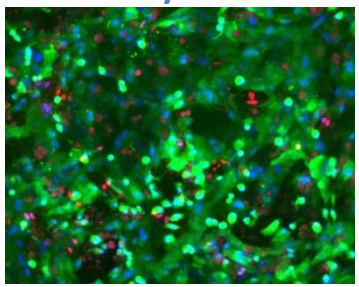
Day 14

Hepatocyte Plane



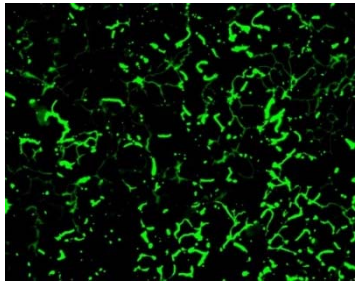
Day 15

Live / Dead

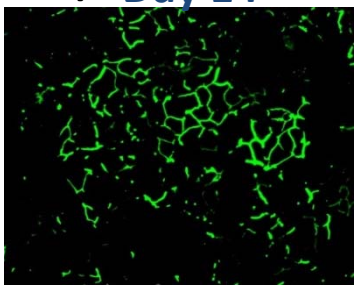


Bile Network Formation (CMFDA)

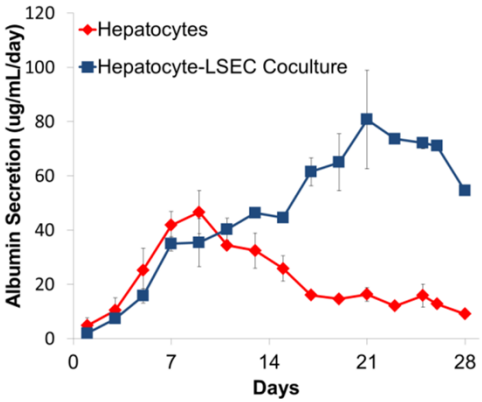
Day 7



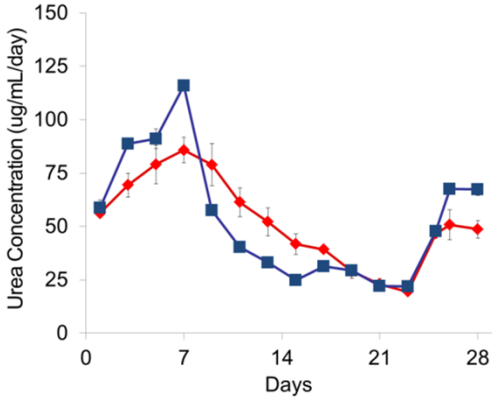
Day 14



Albumin Secretion



Urea Secretion



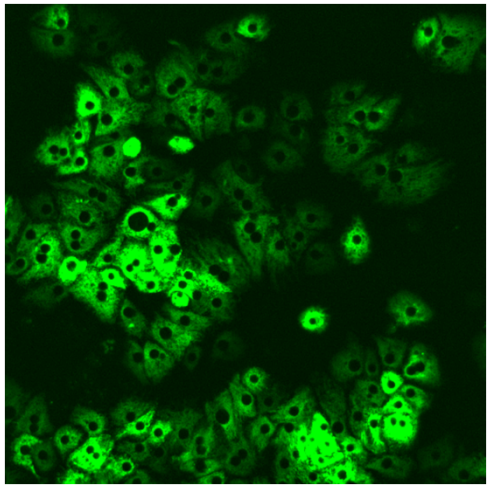
Sentinel Biosensor Development Strategy

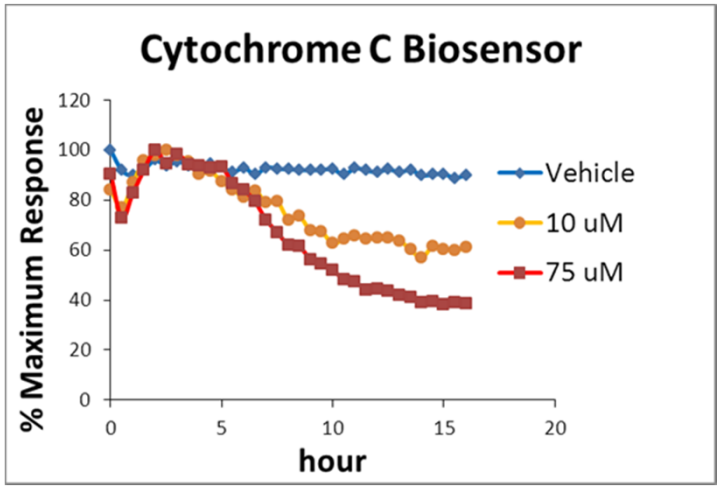
Lentiviral Delivery and then Homologous Recombination in iPSC

Development/Testing	**Initial Platform**	**Final Platform**
HepG2, 1° Human Hepatocytes, Kupffer, Stellate	1° Human Hepatocytes, Kupffer, Stellate	iPSC-derived Human Hepatocytes, Kupffer, Stellate

Example Performance Test – Cytochrome C

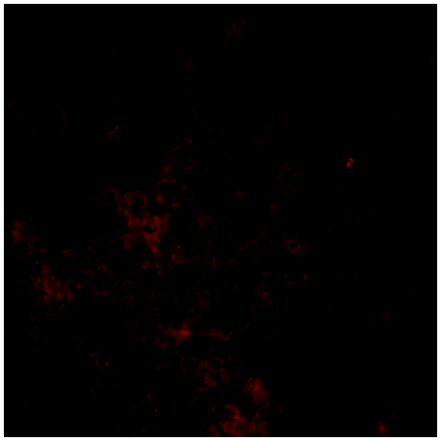
Nefazodone

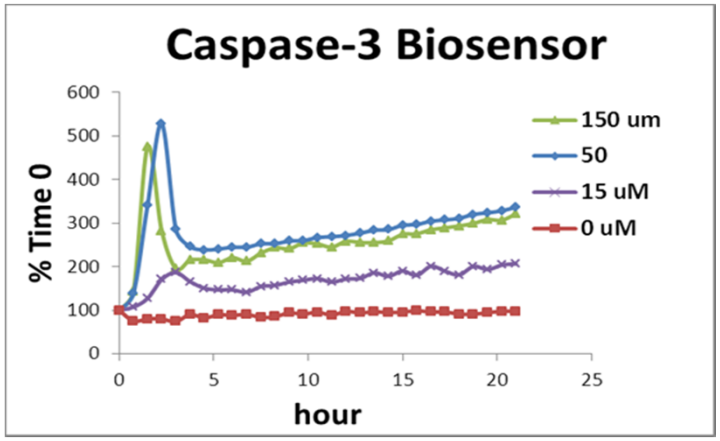




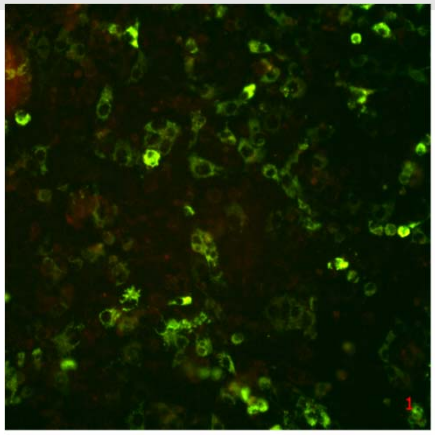
Cytochrome C biosensor releases from the mitochondria in primary human hepatocytes following exposure to 10 µM Nefazadone. False color images

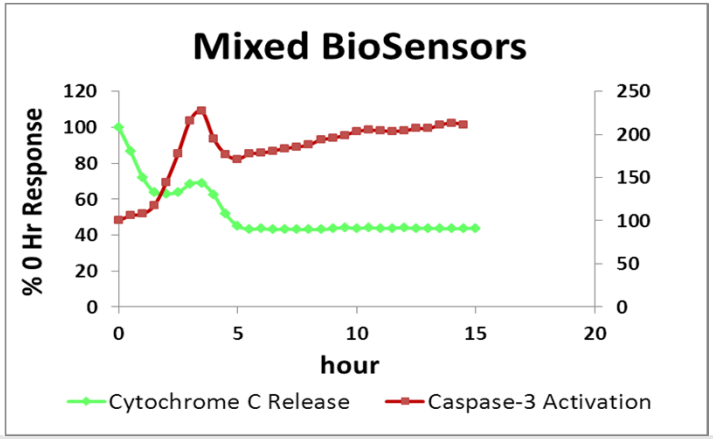
Example Performance Tests (cont)





Casper BG biosensor indicates activation of Caspase-3 in HepG2 cells following exposure to 50 µM Menadione. False color images.

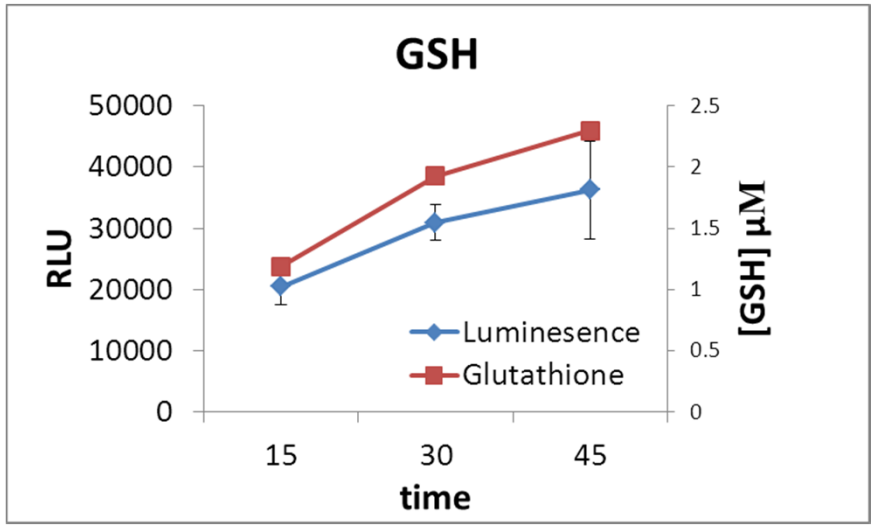


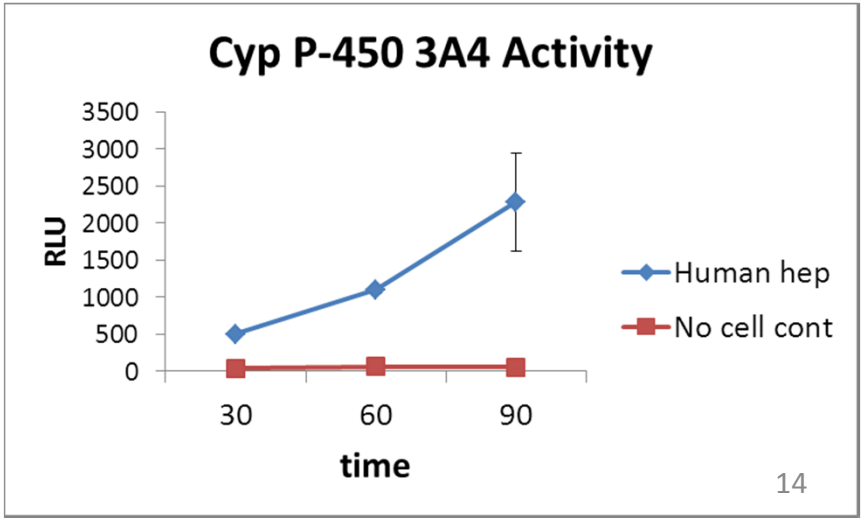


Mixed Cytochrome C & Caspase-3 HepG2 biosensors cells indicates release of cytochrome-c and activation of Caspase-3 following exposure to 50 µM Menadione. False color Green- Cyto C; Red-Caspase 3

Biochemical Characterization Assays

Summary Analysis of Test Results			
Assay	**Method**	**Component**	**Units**
Morphology	Phase microscopy after 18-24 hours	Cell shape, confluency, spreading	none
GSH	Luminescence (Promega)	Glutathione	microMolar
P450	Luminescence (Promega)	CYP 3A4	RLU/mg protein
ECOD t1 – t4	Fluorescence (7-ethoxycoumarin)	CYP1A1, 2B1 and 2B2	picomoles/min/mg protein
EROD t1 – t4	Fluorescence (7-ethoxyresorufin)	CYP1A	picomoles/min/mg protein
Total Cell Protein	Cu2+ reduction (Pierce BCA)	Total cellular protein	mg/well





Microphysiology Database

General Design Principles

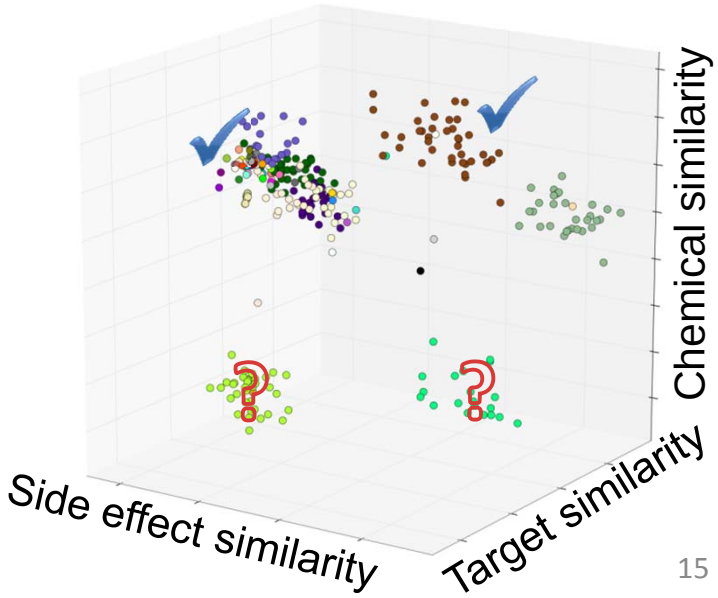
- Cell and device agnostic database design
- Integrating external drug/target data and platform readouts for optimizing reference drug selection
- Linking external drug/target databases through unifying identifiers
- Store platform and cell characterization data for interpreting bioactivity results

Optimizing Liver Reference Drug Set

Break down of 120 reference drugs

- **30 hepatotoxic**
- **30 black-box labeled**
- **30 non-organ toxic**
- **30 other human organ toxic**

Reference drug selection will optimized to increase the coverage of chemical, pharmacological (target), and phenotypic (side-effect) spaces



Web Interface to the Microphysiology DB

Microphysiology Database

Compound | Target | BioAssay | Keyword

liver GO

Advanced Search | Upload Data

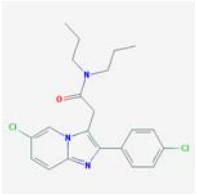
Web User Interface

- Enables users to search data
- Compound, assay, and organ names, as well as commonly used synonyms, target protein names, pathways, therapeutic indications, and external database identifiers can be used for searching

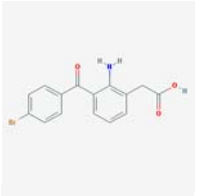
Modify Report:

- Cluster Structures
- Include Targets
- Include BioAssays
- Include Organs
- Save Report
- Save Cmpd List
- Download Data

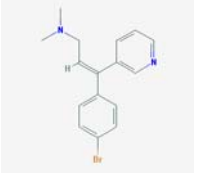
Organ model: liver Search



alpidem
Also known as: Ananxyl, Alpidemum, 82626-01-5
Molecular Formula: $C_{21}H_{23}Cl_2N_3O$
# of Organ Model Readouts: 8 in 3 models
# of BioActivity Readouts: 5 in 1 assays
PubChem CID: 54897



bromfenac
Also known as: Xibrom, Duract, Bromfenacum, Bromfenaco
Molecular Formula: $C_{16}H_{17}BrN_2$
# of Organ Model Readouts: 2 in 1 models
# of BioActivity Readouts: 10 in 1 assays
PubChem CID: 60726



zimelidine
Also known as: Zimelidine, cis-Zimelidine, (Z)-Zimelidine, Zelmid
Molecular Formula: $C_{16}H_{17}BrN_2$
# of Organ Model Readouts: 6 in 2 models
# of BioActivity Readouts: 20 in 6 assays
PubChem CID: 5365247

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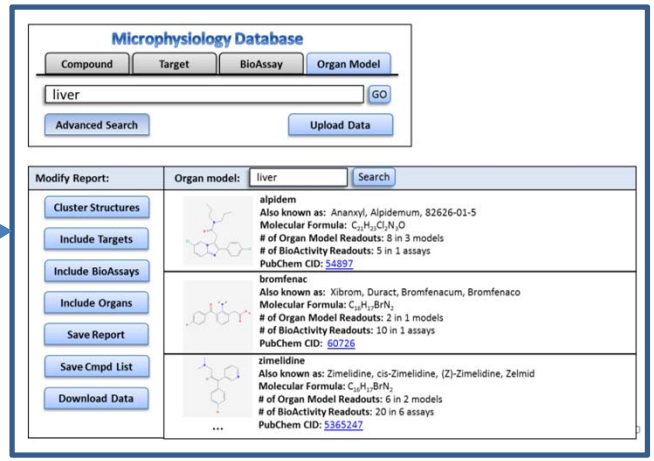
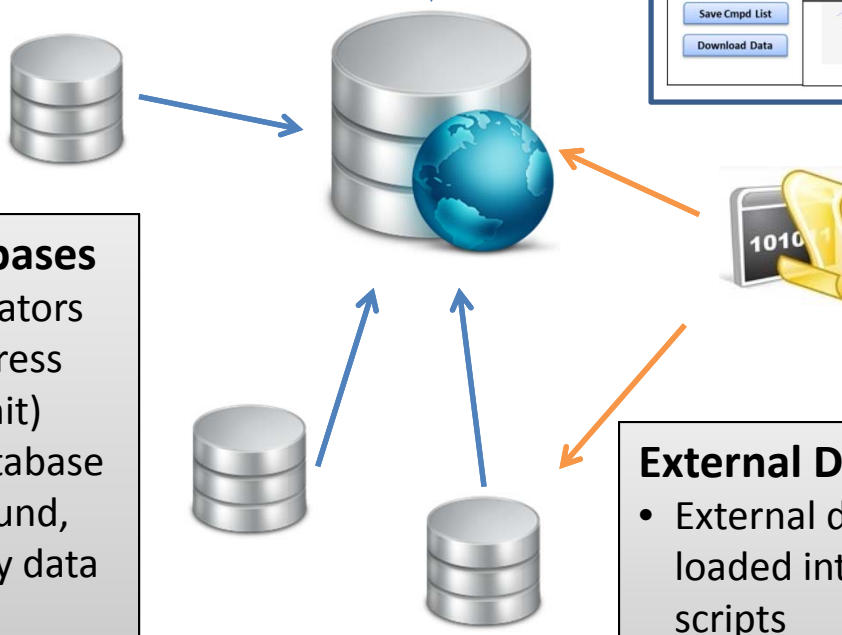
Shared Database Strategy for All Organ Models

Central Database and WUI

- A centralized database consolidates organ specific data
- Data is accessible and searchable through a web user interface





PubChem

SIDER 2
Side Effect Resource

ChEMBL

RCSB PDB
PROTEIN DATA BANK

Organ Specific Databases

- Managed by collaborators using SQL Server Express edition (free, 4GB limit)
- A clone of central database with external compound, target, and bioactivity data is provided
- Graphical interfaces are provided for data entry and querying

External Data

- External data is fetched, filtered, and loaded into database using Python scripts
- Scripts can be run at anytime to update central and organ specific databases when new compounds are available

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Reduce Drug Attrition Rates by

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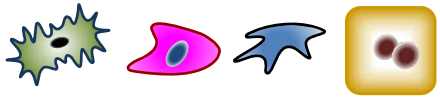
- Predictive Database Modeling

Cell Characterization/ Validation Data

Primary Liver Cells



Sentinel Cells

External Data

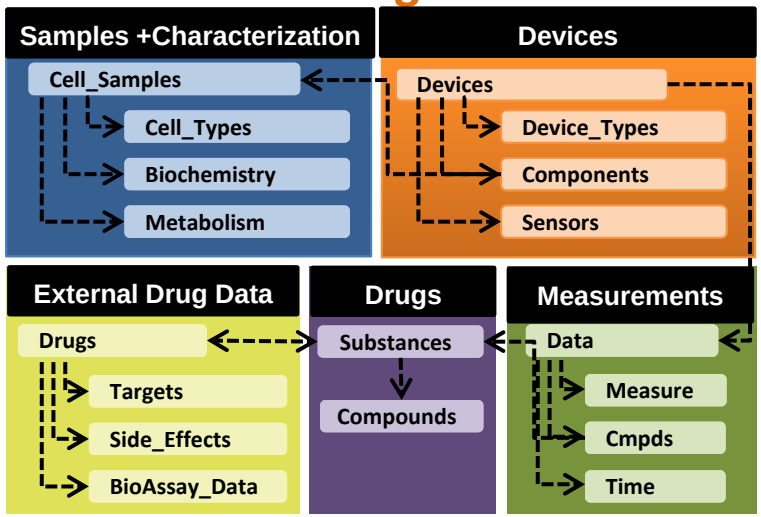
RCSB PDB PROTEIN DATA BANK

ChEMBL

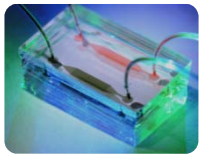
SIDER 2 Side Effect Resource

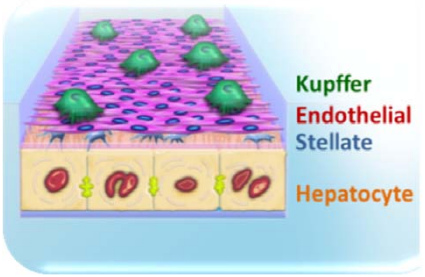
PubChem

Predictive Drug Database



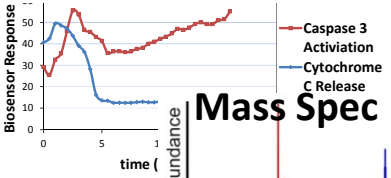
3-D Microfluidic Liver Development



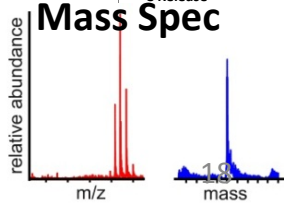


Measurements / Compound Activity

Biosensor/Biochem

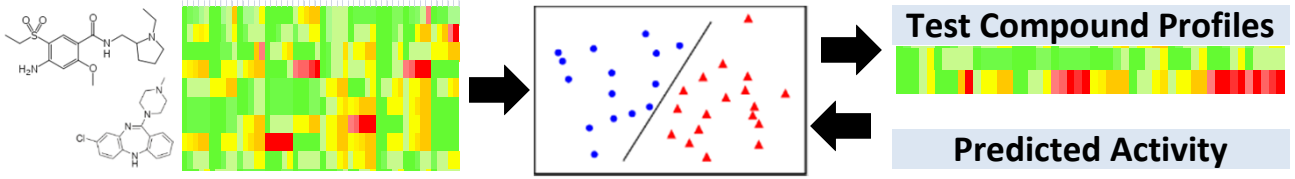


Mass Spec



Predictive Model

Activity profiles for similar compounds (2D, 3D, target, or bioactivity similarity)



Validation of Components and Complete System

Response to Positive Control Drugs

- ❑ **Zonation of O_2 and pH in device**
- ❑ **> 85% cell viability (all 4 cell types)**
- ❑ **Key biochemical assay data at 1 month**
- ❑ **Biosensor "sentinel" cell functions at 1 month**
- ❑ **Bile production at 1 month**
- ❑ **Drug metabolism at 1 month**
- ❑ **ROC curve: > 80% true positive, < 10% false positive**

Acknowledgements

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