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.

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

Cellummen CellCiphr™ Analysis

<table>
<thead>
<tr>
<th>Cell Features</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Loss</td>
<td>Cell Number</td>
</tr>
<tr>
<td>DNA Fragmentation</td>
<td>Nuclear texture</td>
</tr>
<tr>
<td>Nuclear Size</td>
<td>Nuclear Diameter</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Cytochrome C release</td>
</tr>
<tr>
<td>DNA Damage Response</td>
<td>GADD153 expression</td>
</tr>
<tr>
<td>Mitochondrial Function</td>
<td>Mitochondrial potential</td>
</tr>
<tr>
<td>Phospholipidosis</td>
<td>Lysosome expansion</td>
</tr>
<tr>
<td>Steatosis</td>
<td>Neutral lipid accumulation</td>
</tr>
</tbody>
</table>

Rank Order

Similarity Profiles

Safety Alert Prediction

Reference Cpds

Physical Properties

Safety Toxicology

CellCiphr Profiles

Customer Cpds
CellCiphr ROC in 2010

CellCiphr® Outperforms Simpler Assays

Can we do better with Live 3D human models with flow?
Design of the 3D BIOMIMETIC LIVER Device

Inspired by the Liver Acinus

Physiologies to Capture

- Cellular Mechanisms of Action
- Albumin, Urea, LDH leakage, Glucose
- Drug metabolism
- Zonation (O₂, Chemical)
- Bile Production

The Device

Micro-grooved design to create hepatic cords

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

Adams et al. Nat. Rev. Immunology 6, 244–251 (2006)
Biomimetic Liver Platform Overview

**Cell Characterization/Validation Data**
- Primary Liver Cells
  - Sentinel Cells

**External Data**
- PDB
- ChEMBL
- SIDER 2 (Side Effect Resource)
- PubChem

**3-D Microfluidic Liver Development**
- Kupffer Endothelial Stellate
- Hepatocyte

**Broad Platform Goals**
Reduce Drug Attrition Rates by
- Recapitulating Liver Physiology
- Predictive Database Modeling

**Predictive Drug Database**

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

**Test Compound Profiles**

**Predicted Activity**

**Measurements / Compound Activity**
Biosensor/Biochem

**Biosensor Response**
- Caspase 3 Activation
- Cytochrome C Release
- Mass Spec

**Measurements**
- Data
- Measure
- Cmpds
- Time

**Devices**
- Device Types
- Components
- Sensors

**Samples + Characterization**
- Cell_Samples
  - Cell_Types
  - Biochemistry
  - Metabolism

**External Drug Data**
- Drugs
  - Targets
  - Side_Effects
  - BioAssay_Data

**Drugs**
- Substances
  - Compounds

**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
“Sentinel” Cell Reporters

Biosensor expressing cell

Hepatocytes

Bile deposits

Kupffer cells

Endothelial cells

Stellate cells

ECM like Matrix

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
HepG2, 1° Human Hepatocytes, Kupffer, Stellate

Initial Platform
1° Human Hepatocytes, Kupffer, Stellate

Final Platform
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 Nefazodone. False color images
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

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

### Graphs

**GSH**
- RLU vs. time (15, 30, 45 minutes)
- [GSH vs. time graph](#)

**Cyp P-450 3A4 Activity**
- Human hep vs. No cell cont
- RLU vs. time (30, 60, 90 minutes)
- [Cyp P-450 3A4 Activity graph](#)
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 be optimized to increase the coverage of chemical, pharmacological (target), and phenotypic (side-effect) spaces
### Web Interface to the Microphysiology DB

**Microphysiology Database**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Target</th>
<th>BioAssay</th>
<th>Keyword</th>
</tr>
</thead>
<tbody>
<tr>
<td>liver</td>
<td></td>
<td></td>
<td>GO</td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th>Modify Report:</th>
<th>Organ model:</th>
<th>Search</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster Structures</td>
<td>alpidem</td>
<td><img src="#" alt="alpidem structure" /></td>
</tr>
<tr>
<td>Include Targets</td>
<td>Also known as: Ananyxyl, Alpidemum, 82626-01-5</td>
<td></td>
</tr>
<tr>
<td>Include BioAssays</td>
<td>Molecular Formula: C$<em>{21}$H$</em>{23}$Cl$_2$N$_3$O</td>
<td></td>
</tr>
<tr>
<td>Include Organs</td>
<td># of Organ Model Readouts: 8 in 3 models</td>
<td></td>
</tr>
<tr>
<td>Save Report</td>
<td># of BioActivity Readouts: 5 in 3 assays</td>
<td></td>
</tr>
<tr>
<td>Save Cmpd List</td>
<td>PubChem CID: 54897</td>
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</tr>
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</table>

<table>
<thead>
<tr>
<th>Include Organs</th>
<th>bromfenac</th>
<th><img src="#" alt="bromfenac structure" /></th>
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</thead>
<tbody>
<tr>
<td>Also known as: Xibrom, Duract, Bromfenacum, Bromfenaco</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular Formula: C$<em>{16}$H$</em>{17}$BrN$_2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td># of Organ Model Readouts: 2 in 1 models</td>
<td></td>
<td></td>
</tr>
<tr>
<td># of BioActivity Readouts: 10 in 1 assays</td>
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</tr>
<tr>
<td>PubChem CID: 60726</td>
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<table>
<thead>
<tr>
<th>Include Organs</th>
<th>zimelidine</th>
<th><img src="#" alt="zimelidine structure" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Also known as: Zimelidine, cis-Zimelidine, (Z)-Zimelidine, Zelmid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular Formula: C$<em>{16}$H$</em>{17}$BrN$_2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td># of Organ Model Readouts: 6 in 2 models</td>
<td></td>
<td></td>
</tr>
<tr>
<td># of BioActivity Readouts: 20 in 6 assays</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PubChem CID: 5365247</td>
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<td></td>
</tr>
</tbody>
</table>

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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

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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- Substances
  - Compounds

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- Data
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  - Cmpds
  - Time

**Predictive Model**

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

Test Compound Profiles

Predicted Activity
Zonation of O₂ 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
N. Senutovitch,¹,² A. Bakan,² R. DeBiasio,¹ A. Gough,¹,² T. Shun,¹ L. Vernetti,¹,² O. B. Usta,³ A. Bhushan,³ S. S. Bale,³ W. J. McCarty,³ M. Hegde,³ R. Jindal,³ I. Golberg,³ M. L. Yarmush³ and D. L. Taylor¹,²
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