In Vitro Liver Models And Their Applications For NASH

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Non Alcoholic Steatohepatitis: Disease Problem and Unmet Needs

- 1 in 3 adults in the U.S. has non-alcoholic fatty liver disease
- 75% of people with NASH also have type 2 diabetes
- Fastest growing disease in China and India.
- Approximately 50 active programs with 38 distinct therapeutic targets

An ideal in vitro liver model would fulfill various unmet needs:
- Unbiased novel target discovery
- Development of non-invasive translational biomarkers for diagnosis and monitoring disease.
- Understanding/predicting efficacy differences, in stratified sub-populations (Personalized medicine).
- Safety assessment under disease-like conditions.

Source: Catenion analysis based on ADIS database, 2014
Underlying Mechanisms of Steatohepatitis are Complex

 mécanismes de l'atteinte hépatique steatoïde sont complexes

- Mechanisms of Steatosis
  1. ↑ Synthesis of lipids/cholesterol
  2. ↓ β-Oxidation of fatty acids
  3. ↓ Export of lipoproteins
  4. ↑ Uptake of fatty acids

Steatosis → Oxidative Stress → Inflammatory Cytokines → Macrophage/Stellate Activation → Extracellular Matrix Deposition
## Existing in vitro Models: Challenges and Opportunities

<table>
<thead>
<tr>
<th>Species</th>
<th>Cell Type(s)</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human</strong></td>
<td>Hepatocytes</td>
<td>Primary (Healthy/Patient)</td>
</tr>
<tr>
<td></td>
<td>Huh7</td>
<td>Hepatoma</td>
</tr>
<tr>
<td></td>
<td>HepG2</td>
<td>Hepatocellular Ca</td>
</tr>
<tr>
<td></td>
<td>Hepatic Stellate Cells</td>
<td>Healthy/Patient</td>
</tr>
<tr>
<td></td>
<td>LX2 Stellate Cell line</td>
<td>Immortalized</td>
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<tr>
<td></td>
<td>Hepatocytes + Adipose Cells</td>
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<tr>
<td></td>
<td>Huh7 + LX2</td>
<td></td>
</tr>
<tr>
<td><strong>Canine</strong></td>
<td>Hepatocytes</td>
<td>Primary</td>
</tr>
<tr>
<td><strong>Rat</strong></td>
<td>Primary Hepatocytes</td>
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<td></td>
<td>H4IE</td>
<td>Immortalized</td>
</tr>
<tr>
<td></td>
<td>H4IEC3</td>
<td>Immortalized</td>
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<tr>
<td></td>
<td>PAV-1</td>
<td>Immortalized</td>
</tr>
<tr>
<td><strong>Mouse</strong></td>
<td>RAW 264.7 Macrophages and AML-12 Cell co-cultures</td>
<td>Immortalized</td>
</tr>
</tbody>
</table>

### Challenges of existing models employing static flat-plate cell cultures:
- Dedifferentiation and loss of CYP activity.
- Non-physiological levels of glucose and insulin and loss of insulin sensitivity.
- Altered baseline inflammatory state.
- Hypoxia-reperfusion on media change.
- Non-relevant drug and metabolite concentration profiles

### Opportunities for improvement:
- Organotypic approaches (3-D, heterotypic cell interactions, flow).
- Physiological media formulations and drug concentrations based on clinical pharmacokinetics.
- Use of Translational biomarkers.
- Big data –omic approaches.
Recreating Physiological Milieu and Parameters in a 3D Culture Configuration

- 3D cell configuration - modeled on sinusoid with hepatocytes ± non-parenchymal cells.
- Simultaneous perfusion and hemodynamics - allows control of drug, nutrient and oxygen gradients
- Effluent and cells can be assessed from top and bottom separately.

Adapted from: Nature Reviews Immunology 14, 181–194 (2014)


Hepatocytes Plated

Restoration of Biology

Treatment

1. RNA-Seq Analysis
2. Functional Endpoints e.g. MTT, Imaging, CYP Assays
3. Secreted Biomarkers e.g. Albumin, Cytokines, FGF19
Liver-like Polarized Morphology and Function Maintained Over Time

- **TIGHT JUNCTIONAL PROTEIN**
  - E-cadherin HNF4α

- **SURFACE ANTIGENIC ENZYME**
  - CD26, Draq5

- **BILIARY EFFLUX TRANSPORTER**
  - MRP2 HNF4α

- **SURFACE GLYCOPROTEIN**
  - CD81, Draq5

**ALBUMIN SECRETION (ANABOLIC)**
- HemoShear
- Static

**UREA SECRETION (CATABOLIC)**
- HemoShear
- Static

**CYTOCHROME P450 ACTIVITY**
- CYP1A2
- CYP2B6
- CYP3A4/5
- CYP2C9
- CYP2D6

**TRANSPORTER ACTIVITY**
- CDFDA → CDF

Dash et al SOT 2013, Marukian et al AASLD 2013
Drug Responses Exhibited at Clinically Relevant Concentrations

- Efficacy and toxicity responses seen at concentrations that match clinical therapeutic exposures.
- Over 30 drugs assessed for mechanistic differences using transcriptomics. (NIH SBIR Award R44 DK091104-02)

Rumack-Matthews nomogram for serum concentration thresholds for clinical treatment of Acetaminophen poisoning.

Figler et al AASLD 2015.
Insulin sensitivity allows culture in a close to physiologic milieu and altered disease-like steatotic phenotype under hyperglycemic, hyperinsulinemic conditions.

Applications of a Physiologically Responsive Liver Model

After demonstrating that the system maintained differentiated liver phenotype as evidenced by polarized morphology, liver specific functions, drug metabolizing enzyme and transporter activity and responsiveness to insulin, we tested the model for the following applications:

1. Assessing on-target and off-target pharmaco-toxicology of drugs at clinically relevant concentrations.
2. Distinguishing transcriptomic signatures of various phenotypes of drug induced liver injury (DILI).
3. Studying underlying mechanisms of drug induced steatohepatitis that could help understand potential NASH targets.
4. Developing a lipotoxic model with milieu mimicking metabolic disease.
Assessing On-target Pharmacology of Obeticholic Acid

- Strongly induced FGF19 in hepatocytes, both at a gene and protein level, confirming a direct hepatic effect in addition to the widely appreciated FGF19 loop through the gut.

- CYP7A1 was the most down-regulated differentially expressed gene in the transcriptome, with simultaneous down-regulation of the bile synthesis pathway genes.

Sanyal, Oral presentation AASLD 2015
Pathway analysis and scoring confirmed beneficial effects of obeticholic acid on reducing steatotic indices and inflammatory signaling.

Functional CYP assays revealed that obeticholic acid suppressed CYP1A2 and CYP3A4 activity.
Distinguishing Drug Induced Steatohepatitis Signatures From Other Forms of Drug Induced Liver Injury

- Transcriptomic analysis allowed us to characterize distinct signatures for different drugs having different DILI phenotypes.
- Assays like Nile Red (neutral lipid) and secreted protein biomarkers in effluent media were confirmatory functional endpoints that defined the steatohepatitic phenotype.

Figler et al AASLD 2015.
Understanding Mechanisms and Potential Targets of Steatohepatitis

- Differential analysis of transcriptomic signatures for different drugs causing steatohepatitis versus those causing NASH may offer insights into mechanisms and targets.

Dash et al. EASL 2016.
Liver Models to Recapitulate Metabolic Disease Spectrum

- **Healthy Milieu**: Healthy Milieu
- **Steatotic Milieu**: Steatotic Milieu (Glucose, Insulin)
- **Lipotoxic Milieu**: Lipotoxic Milieu (Steatotic + Fatty Acids)
- **Lipotoxic Milieu + TNF**: Lipotoxic Milieu + TNF

### Graphs

- **Hepatic Triglycerides**
- **Lipid Accumulation**
- **Inflammatory Cytokines**

### Data

- **Relative Triglycerides (FOLD)**
- **Relative Nile Red (FOLD)**
- **CXCL10 (pg/ml)**

### Table

<table>
<thead>
<tr>
<th>Condition</th>
<th>Glucose</th>
<th>Insulin</th>
<th>FFA</th>
<th>TNF</th>
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</thead>
<tbody>
<tr>
<td>Healthy Milieu</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steatotic Milieu</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lipotoxic Milieu</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lipotoxic Milieu + TNF</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>

- **Lipotoxic metabolic disease model has Kupffer cells and stellate cells added on opposite side of the membrane**
Ongoing Validation of Drug Responses in the Advanced Lipotoxic Liver System

- In the advanced lipotoxic liver system, OCA
  - Reduced ALT levels (a clinical biomarker for NASH)
  - Promoted a robust increase in downstream targets of FXR signaling, including FGF19
  - Reduced several markers of inflammation
  - Reduced markers of fibrosis

**p<0.005, *p<0.05 relative to VEH**
Conclusions and Future Directions

• The physiologically responsive liver model allows assessment of on-target and off-target pharmaco-toxicology of drugs at clinically relevant concentrations.

• Comparative analysis of transcriptomic responses of drug induced steatohepatitis, lipotoxic NASH-like conditions and drugs that impact NASH could help identify and understand potential NASH targets.

• Ongoing/Future activities include:
  • Benchmarking signatures against clinical samples.
  • Analysis of non-parenchymal cell response within system.
  • Lipidomic analysis to gain a better understanding of lipid fractions under lipotoxic milieu and how they correlate with transcriptomics.
  • Characterization of translatable functional responses such as histology and extracellular matrix composition measurements.
  • Comparative analysis of drug response under healthy versus lipotoxic conditions and stratified patient derived hepatocytes versus human hepatocytes could provide additional insights about useful applications of this system.
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• R44GM109539: Development of an iPSC-derived human hepatocyte platform for drug development.

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