Illuminating Human Function
Microphysiological Flux Balance Platform Unravels the Dynamics of Drug Induced Steatosis

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Drug development is a long sisyphean process costing $3-12 billion per drug. 

- 90% of drugs fail in clinical studies
- 10% of drugs fail after regulatory approval
- 270 drugs withdrawn and 308 discontinued since 1951; most due to adverse events

- No Information is gained from failure
- Animals don’t replicate human response

1. PhRMA, 2006 Industry Focus (2004 global numbers)
Animal Models Lack Predictivity

Over 70% of the drugs toxic to humans are not toxic to animals and vice versa. Drug efficacy shows similar trends.

- Thalidomide causes birth defects in humans but safe in rodents \(^1\)
- Aspirin causes developmental toxicity in rodents but safe in humans \(^2\)

Microwells protect the micro-tissues from shear forces, while nutrient gradients push cells toward metabolic zonation.
Current Organ on Chip technology creates healthy or diseased 3D human organs in generic microfluidics, capturing human genetics and physiology.

- **Low throughput** technology
- Limited to **end-point data**
- No information about mechanism of action
Real Time Monitoring of Oxygen Consumption

Tissue embedded micro-sensors permit continuous, focus-independent, *real time* monitoring of oxygen consumption.

![Graph showing oxygen consumption over time with different concentrations of Thapsigargin](image)

- **Oxygen (% Air)**
  - Control
  - 10 µM
  - 20 µM
  - 30 µM
  - 50 µM
  - 80 µM
  - 115 µM

- **Time (hours)**
  - 0 to 24

**Thapsigargin (chemotherapeutic)**

**Cells / Microsensors**
Increasing throughput is restricted due to the limitations of chip technology, culture systems, and detection methods.

System assembly and monitoring hold significant manual labor and cause bias.
Valproic acid (VPA) is primarily used to treat epilepsy, bipolar disorder and migraines. Exact mechanism of action is unknown.

➢ Valproate has been associated to induce fatty liver in both rodents and humans

➢ VPA toxicity is suggested to be due to metabolites generated at high doses suppresses β-oxidation through PPARα

Valproate (anti-convulsant)
Valproate (anti-convulsant)

- Valproyl-CoA is undetectable in Valproate treated patient's serum or urine sample \(^1\).
- Valproate induces damage in patients only months to years following initial exposure.
- Valproate induce steatosis even without cell death in vitro.
- Valproate is associate with hyperammonemia in children and elderly patients leading to encephalopathy.

Vertical section offers a time-dependent view of toxicity

- 30 mM
- 5 mM
- 20 mM
- 1 mM
- 15 mM
- Control
- 10 mM
- 7 mM
- 6 mM

**Time (hrs)**

**Oxygen (%Air)**

**Viable Fraction**

**Valproate (mM)**

- 24 h $T_{C50} = 27$ mM
- 42 h $T_{C50} = 14$ mM

**TC50 (mM)**

- Real $T_{C50} = 13$ mM
Horizontal sections segregate direct from indirect effects, and analytically derive exposure limit (LEL).

Time-Dependent View of Toxicity

TTO = 6-36 hours

Exposure Limit LEL = 0.3 mM
No proliferation

Steady state

Limited lipids in media

Glucose $\rightarrow$ 2 Lactate + 2 ATP

Glucose + 6 $O_2$ $\rightarrow$ 6 $CO_2$ + 32 ATP

Glutamine $\rightarrow$ Lactate + 3 ATP

Glucose $\rightarrow$ DNA

Glucose $\rightarrow$ Fatty Acids

Fatty Acids + $O_2$ $\rightarrow$ ATP

Glucose utilization in 3D perfused human liver organoid

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Metabolic Flux (nmol/min/10^9 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration</td>
<td>0.093</td>
</tr>
<tr>
<td>Glycolysis</td>
<td>1.693</td>
</tr>
<tr>
<td>Lipogenesis</td>
<td>3.547</td>
</tr>
<tr>
<td>ATP Production</td>
<td>6.373</td>
</tr>
</tbody>
</table>
Metabolic Microsensor Array

Microfluidic sensor array for glucose, lactate and glutamine. Real-time measurements.

![Graph showing sensor readings for various metabolites over time]

- Glutamate
- Glutamine
- Glucose
- Lactate
- Temperature

![Chemical reactions for metabolite detection]

- Glutamine to Glutamate
- Glutamate to 2-Oxoglutarate
- Glucose to Gluconic acid
- Lactate to Pyruvate
- Non-specific Oxidation

Temperature (°C)
Sensor (nA/cm²)

Time (min)
Valproate Metabolic Analysis

Cells shift from glycolysis toward lipid production in minutes, suggesting a non-transcriptional mechanism.
Valproate Metabolic Analysis

❖ Viability >95%
❖ ATP production >84% of untreated cells
❖ 31% increase in glutamine (hyperammononemia) – 15 hours
❖ 14% increase in lipid synthesis (steatosis) – 40 hours

➢ Response kinetics demonstrate that valproate induced steatosis occurs through non-transcriptional mechanism.
Simple is Better.

Mechanistic data allows industry to learn from failure, cutting time and costs of drug development

➢ Simple integration into lab routines
➢ Early detection of toxicity
➢ Unique models of disease: diabetes, heart attack, stroke

1. European Commission, SME Seal of Excellence (Feb. 2019)
Cloud-Based Metabolic Fingerprinting

Metabolic data structures permits cloud-based machine learning of new mechanism of action.
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Stavudine Metabolic Analysis

- Viability >99%
- Stavudine shows a transient lipogenesis and global metabolic suppression
- Transient 5% increase in lipid synthesis – **10 hours**
- 36% decrease in lipid synthesis (*β-ox inhibition*) – **30 hours**