Illuminating Human Function

Microphysiological Flux Balance Platform Unravels the Dynamics of Drug Induced Steatosis

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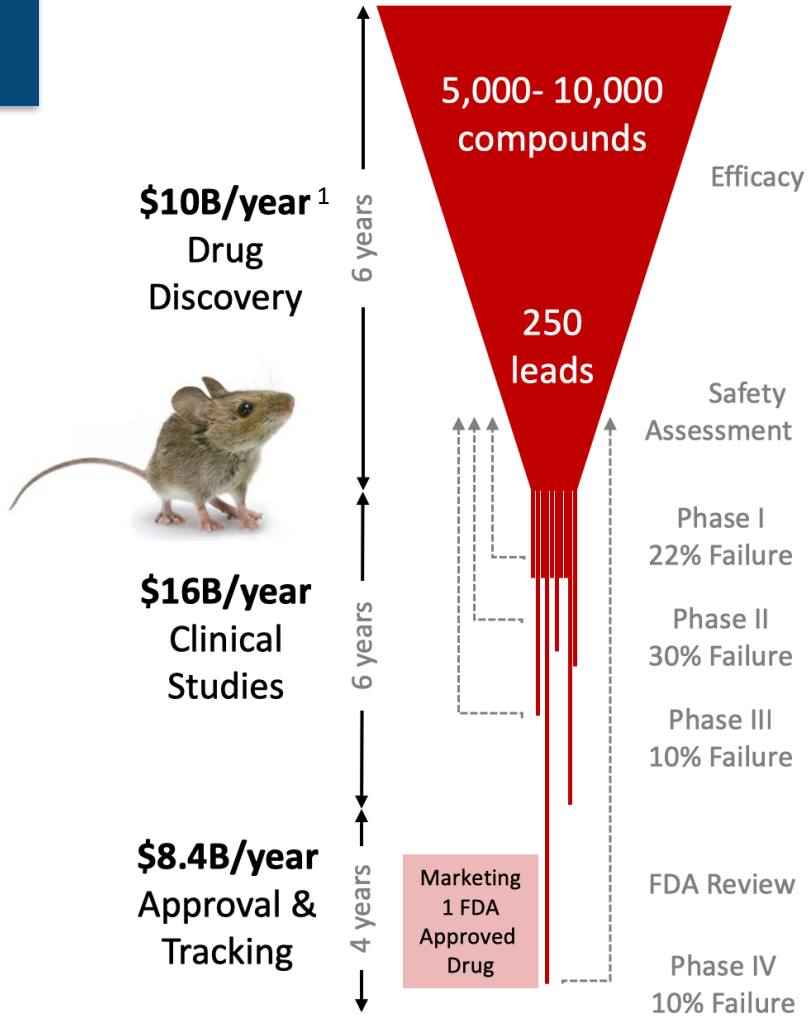
Drug Development as a Gamble

Drug development is a long sisyphean process costing $3-12 billion per drug [2]

- ➢ 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- ➢ 270 drugs withdrawn and 308 discontinued since 1951; most due to adverse events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

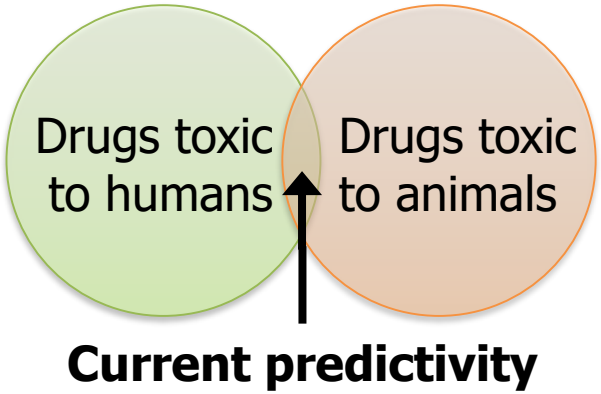


1. PhRMA, 2006 Industry Focus (2004 global numbers)
2. AstraZeneca (2013), ""Quantitative Decision-Making in Drug Development"

Animal Models Lack Predictivity

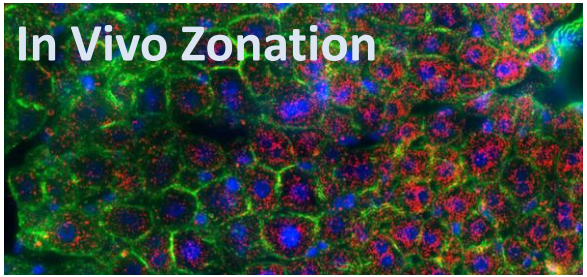
Over 70% of the drugs toxic to humans are not toxic to animals and vice versa. Drug efficacy shows similar trends.

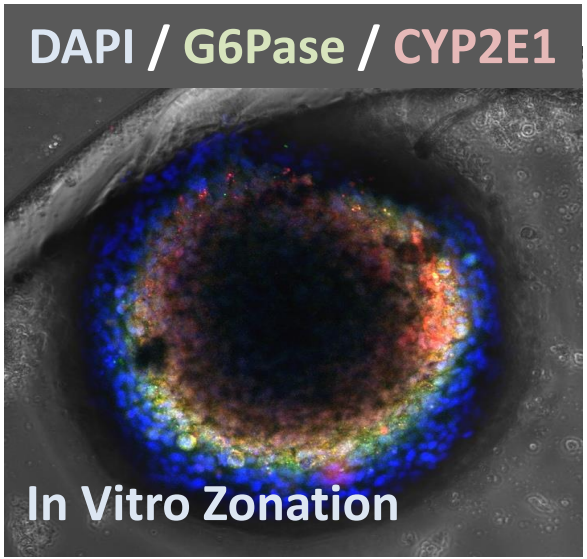
- Thalidomide causes birth defects in humans but safe in rodents [1]
- Aspirin causes developmental toxicity in rodents but safe in humans [2]

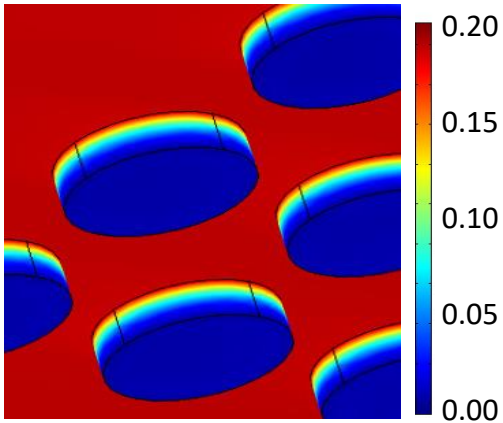


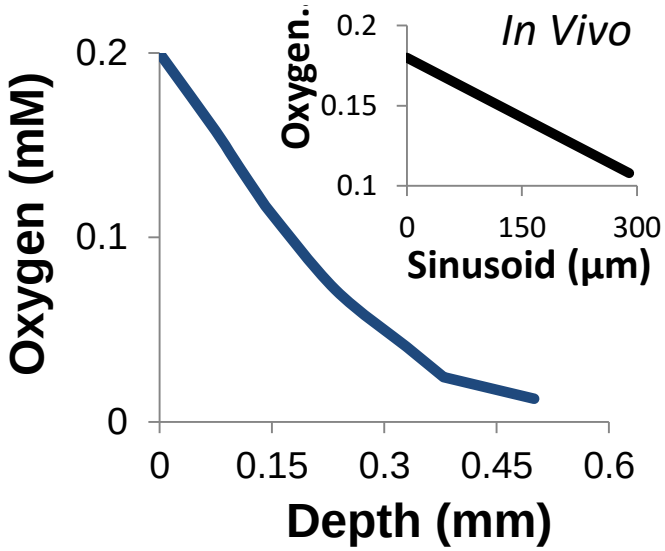
1. Kim & Scialli (2011); 2. Kushima et al. (2007)

Human Liver - Metabolic Zonation on Chip





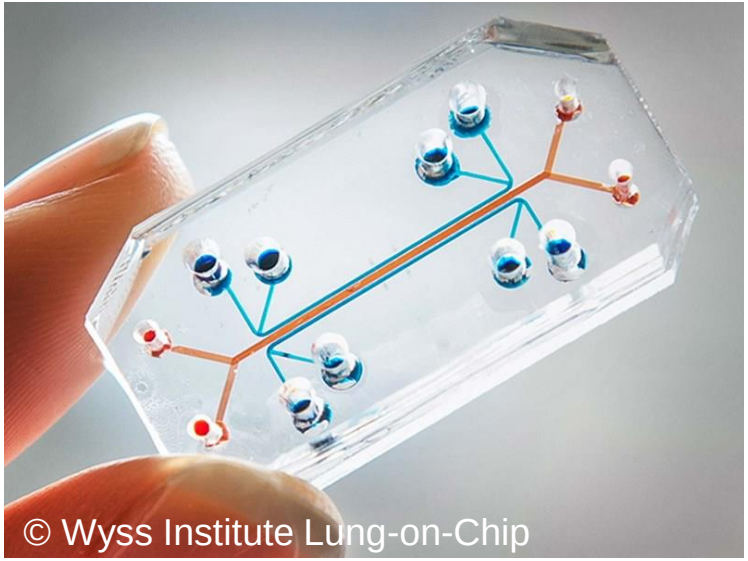




Microwells protect the micro-tissues from shear forces, while nutrient gradients push cells toward metabolic zonation

Current Organ on Chip Approach

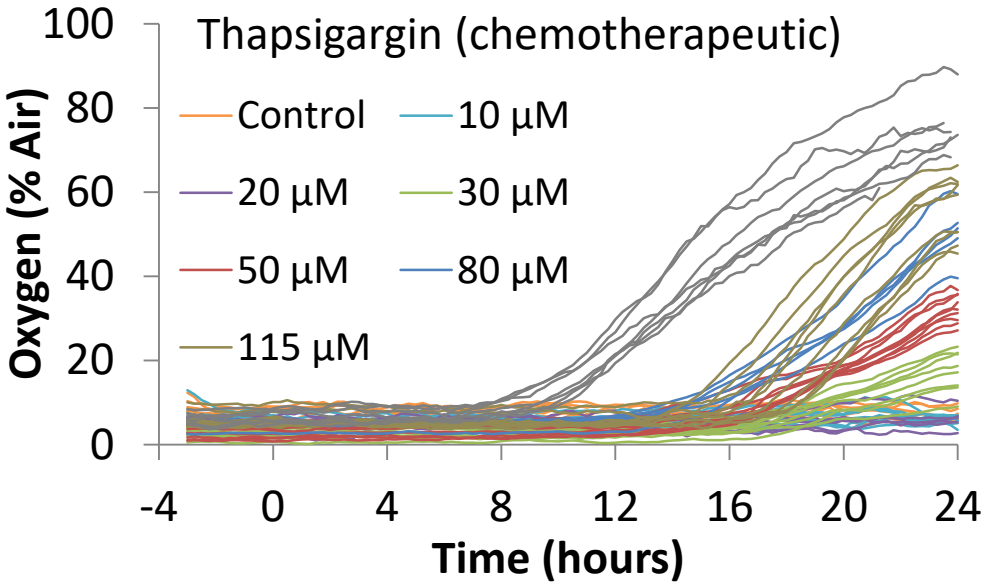
Current Organ on Chip technology creates healthy or diseased 3D human organs in generic microfluidics, capturing human genetics and physiology.

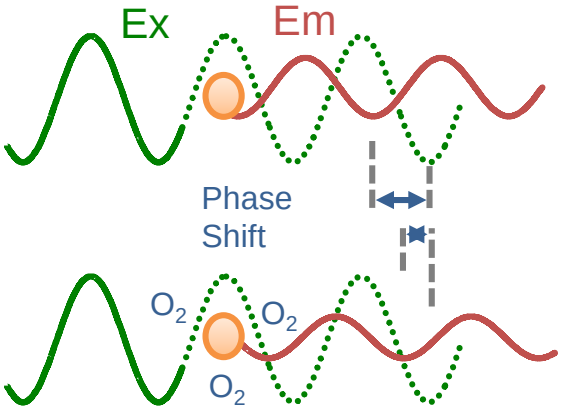


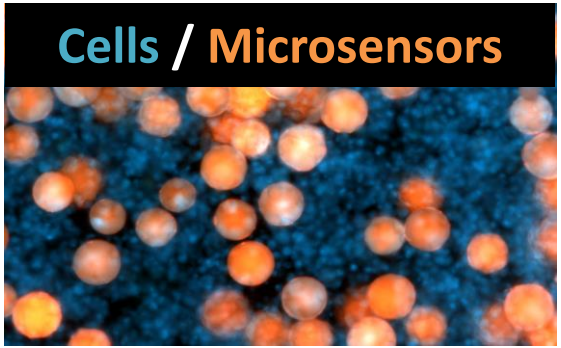
- Low throughput technology
- Limited to end-point data
- No information about mechanism of action

Real Time Monitoring of Oxygen Consumption

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

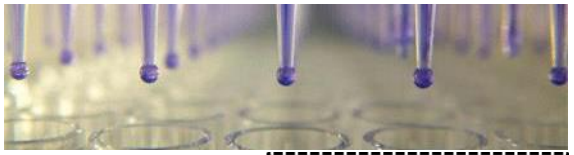


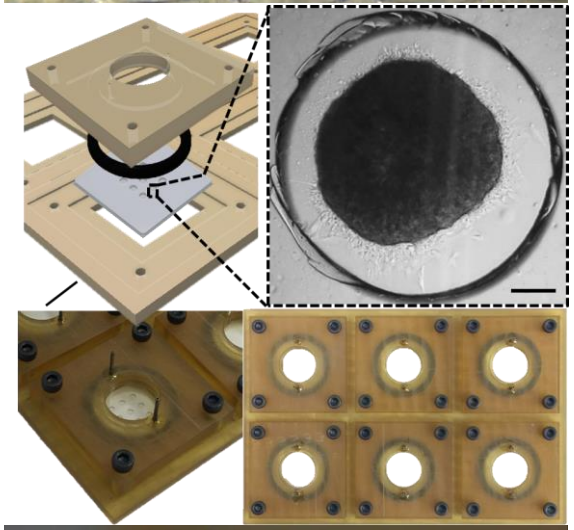


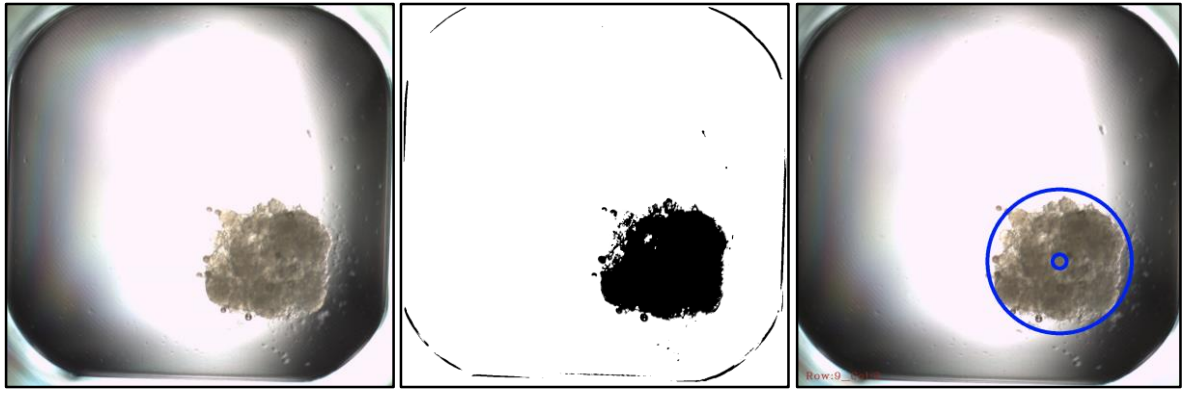


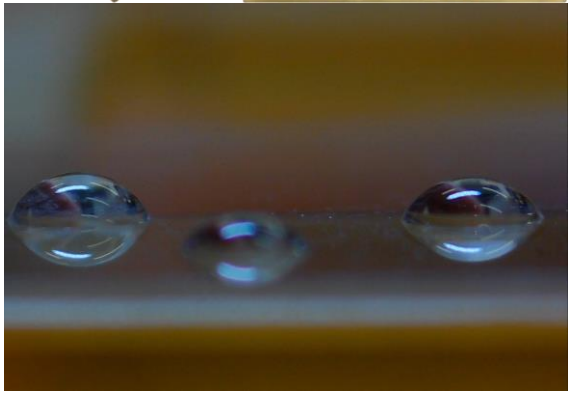
Upscaling the Technology

- Increasing throughput is restricted due to the limitations chip technology, culture systems and detection methods
- System assembly and monitoring hold significant manual labor and cause bias





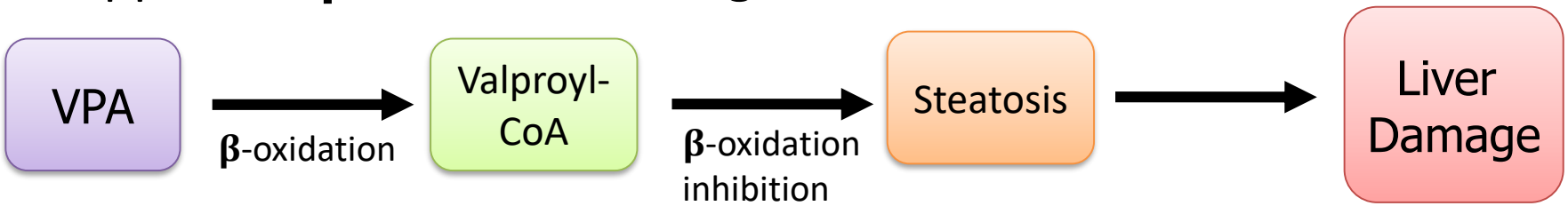




Valproate (anti-convulsant)

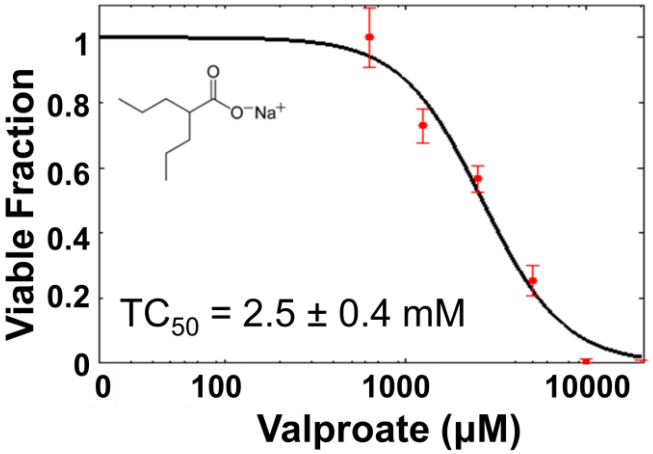
Valproic acid (VPA) is primarily used to treat epilepsy, bipolar disorder and migraines. Exact mechanism of action is unknown.

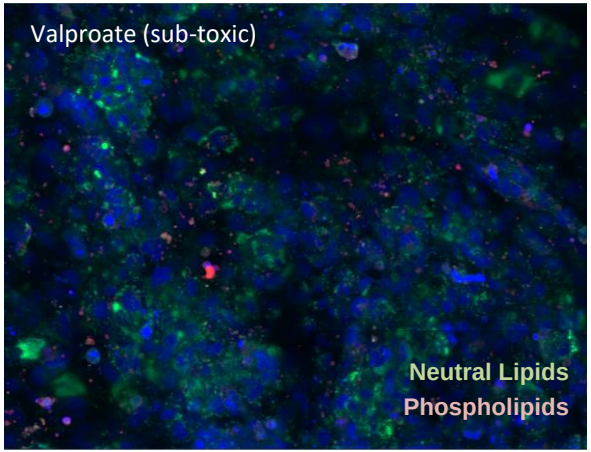
- Valproate has been associated to induce fatty liver in both rodents and humans
- VPA toxicity is suggested to be due to metabolites generated at **high doses** suppresses β-oxidation through PPARα



Valproate (anti-convulsant)

- 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 hyperamonemia in children and elderly patients leading to encephalopathy

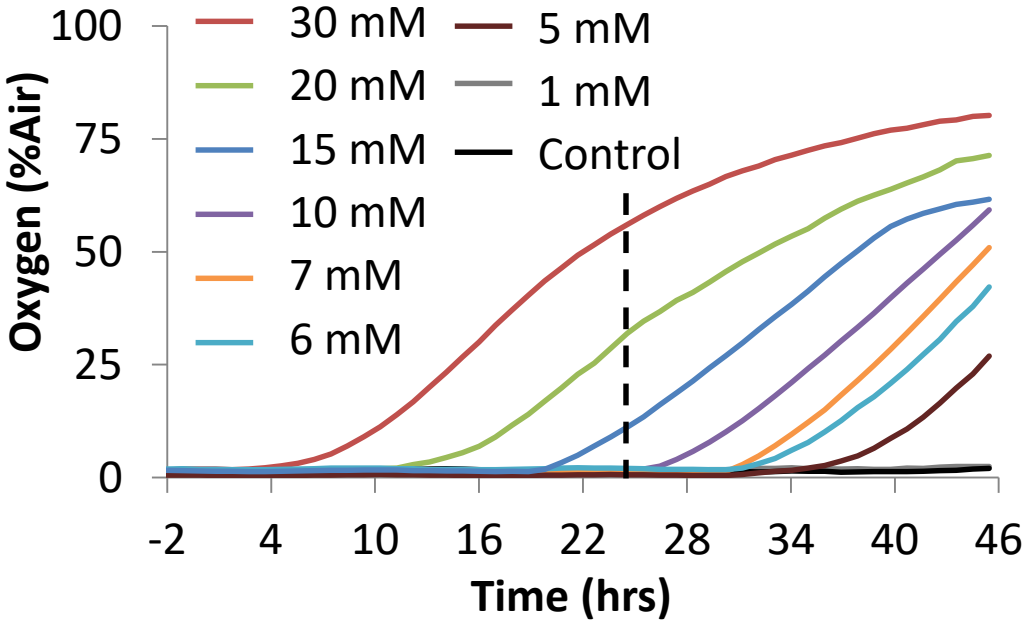


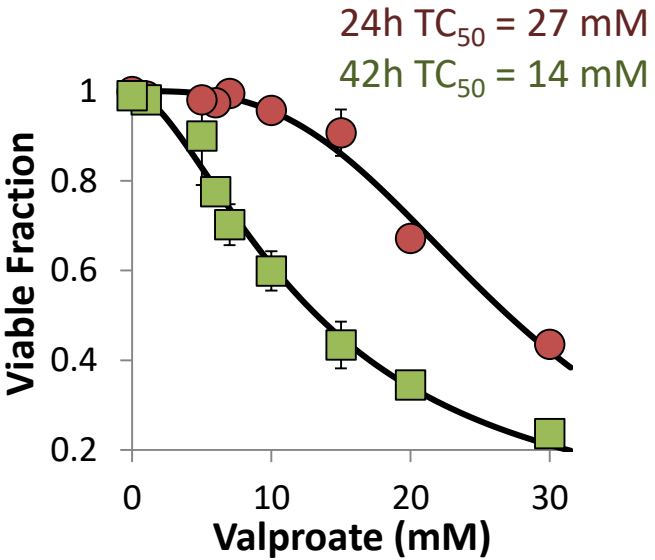


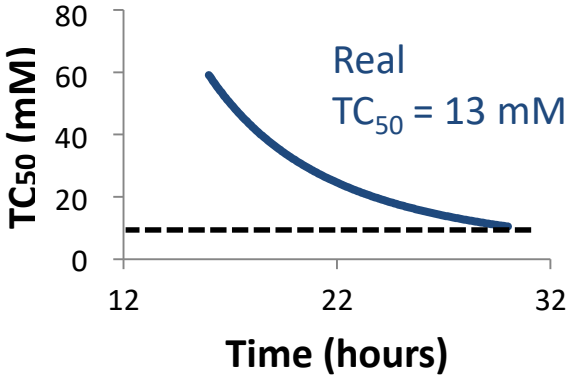
1. D. B. McLaughlin et al, 2000; Y. Ghodke-Puranik et al, 2013.

Time-Dependent View of Toxicity

Vertical section offers a time-dependent view of toxicity

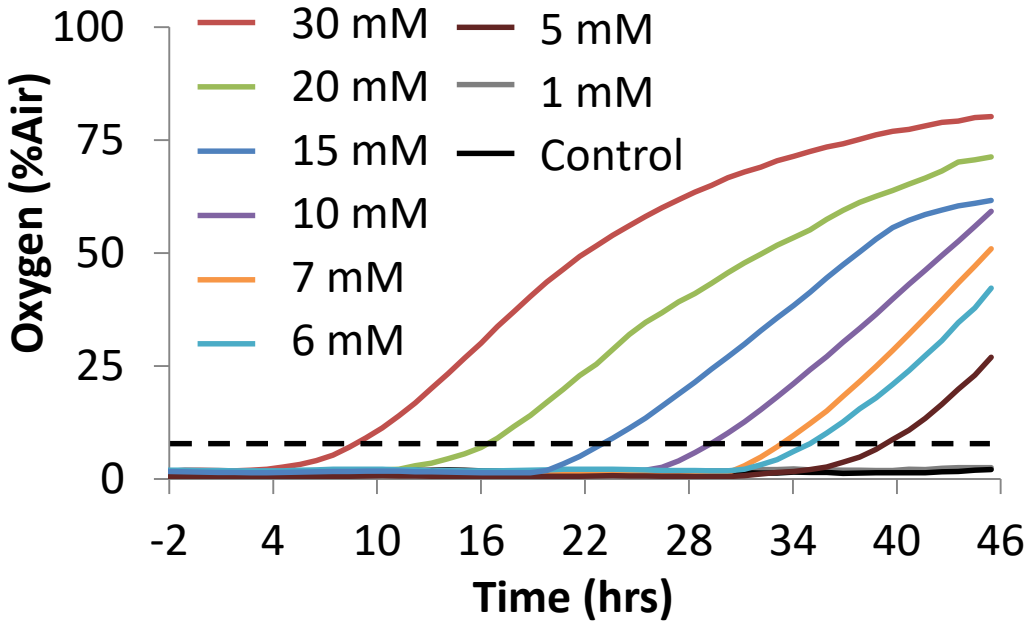


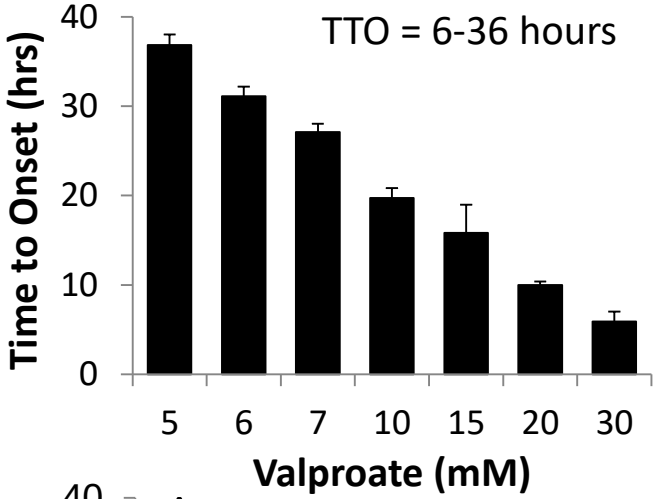


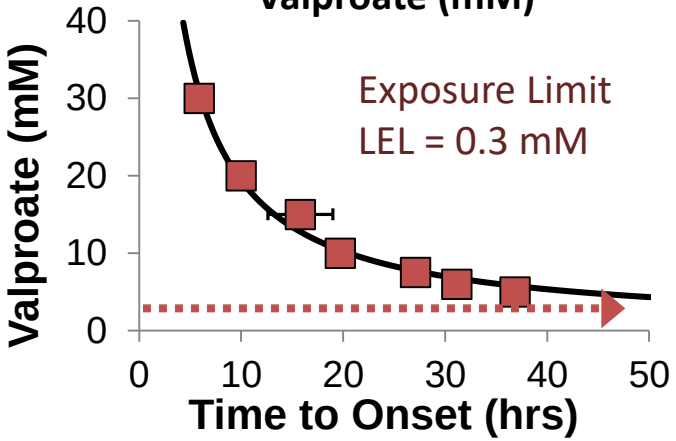


Time-Dependent View of Toxicity

Horizontal sections segregate direct from indirect effects, and analytically derive exposure limit (LEL)







Metabolic Fluxes as Predictors of Toxicity

- No proliferation
- Steady state
- Limited lipids in media

Glucose → 2 Lactate + 2 ATP

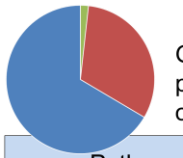
Glucose + 6 O_2 → 6 CO_2 + 32 ATP

Glutamine → Lactate + 3 ATP

~~Glucose → DNA~~

Glucose → Fatty Acids

~~Fatty Acids + O_2 → ATP~~

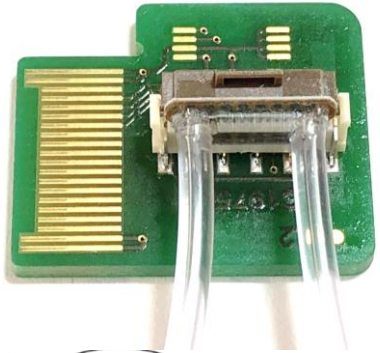


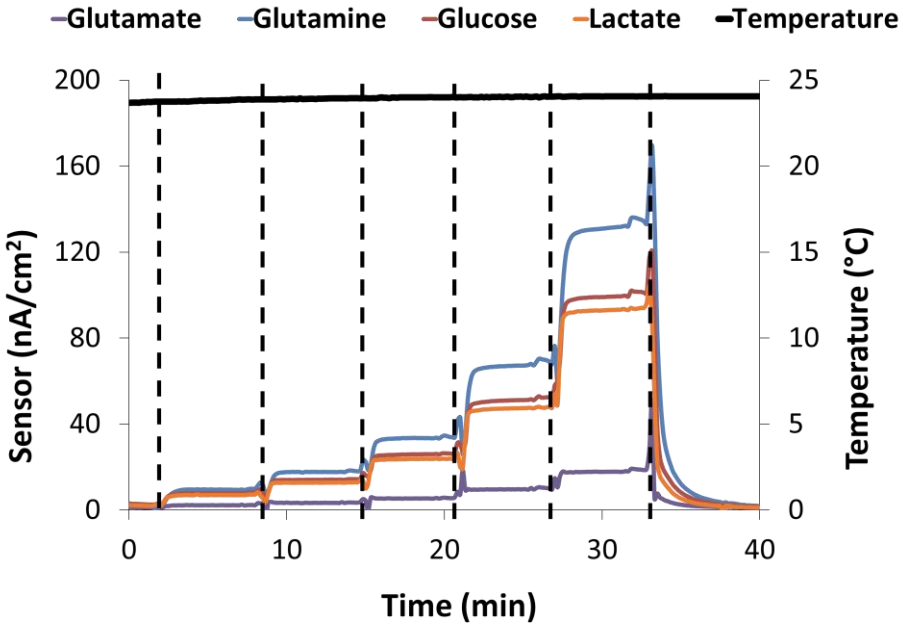
Glucose utilization in 3D perfused human liver organoid

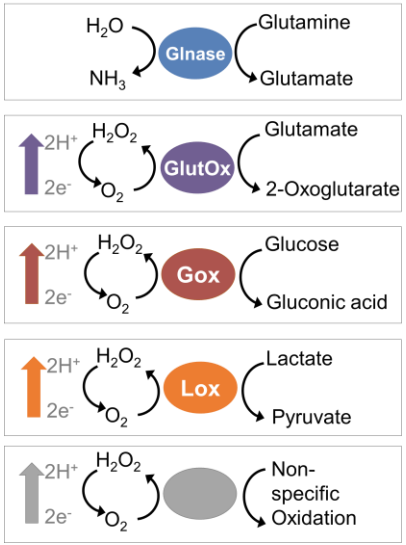
Pathway	Metabolic Flux (nmol/min/10^6cells)
Respiration	0.093
Glycolysis	1.693
Lipogenesis	3.547
ATP Production	6.373

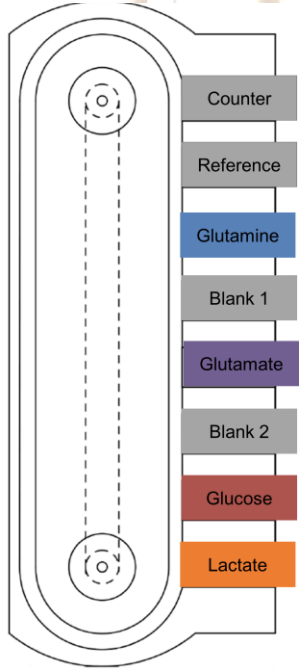
Metabolic Microsensor Array

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

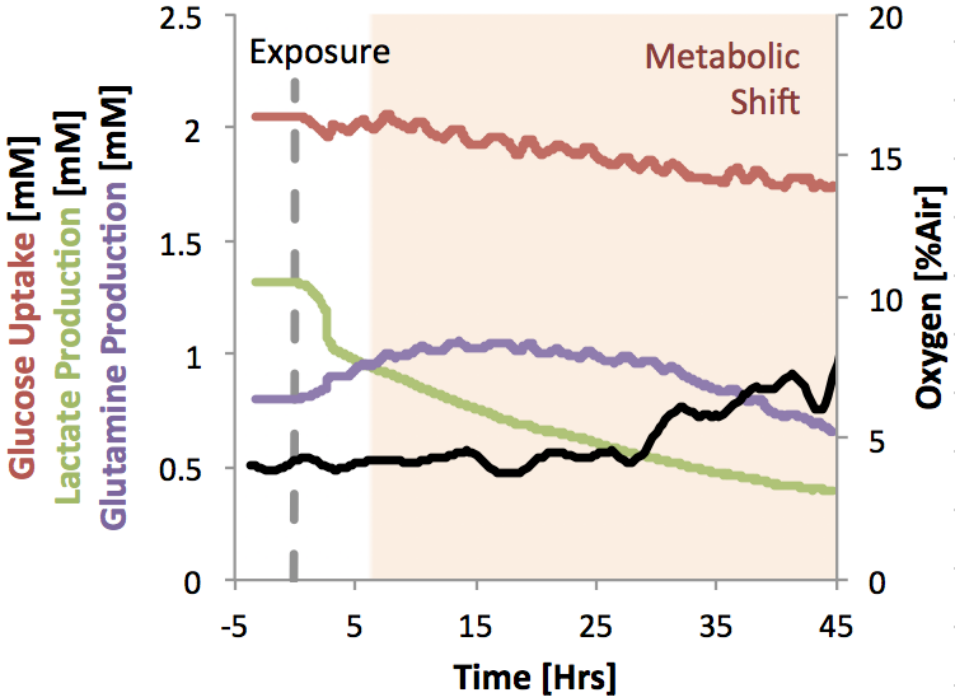


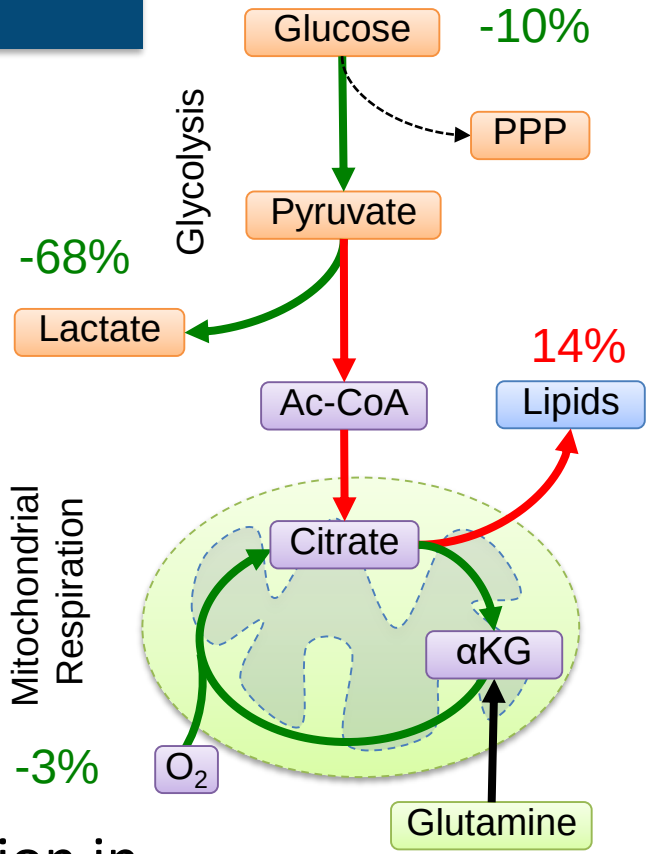






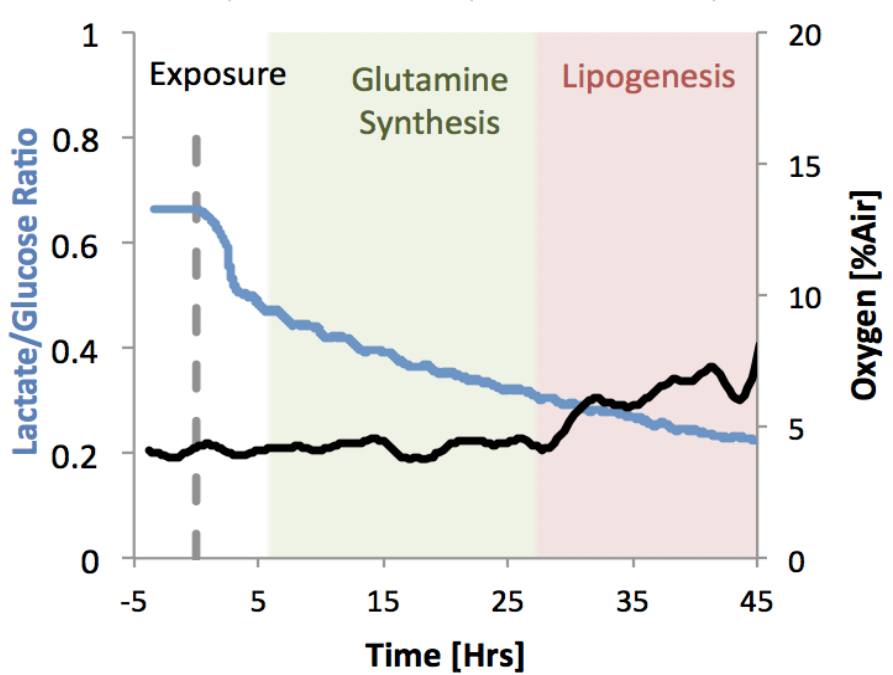
Valproate Metabolic Analysis



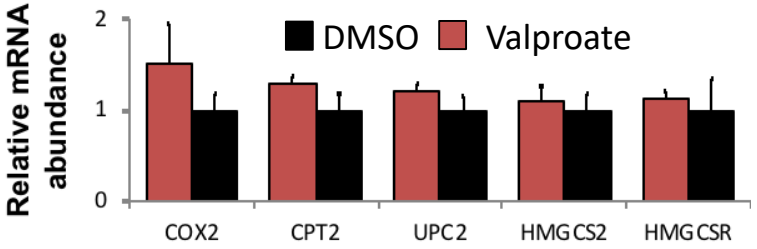


Cells shift from glycolysis toward lipid production in minutes, suggesting a non-transcriptional mechanism.

Valproate Metabolic Analysis



- Response kinetics demonstrate that valproate induced steatosis occurs through non-transcriptional mechanism.



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

Simple is Better.

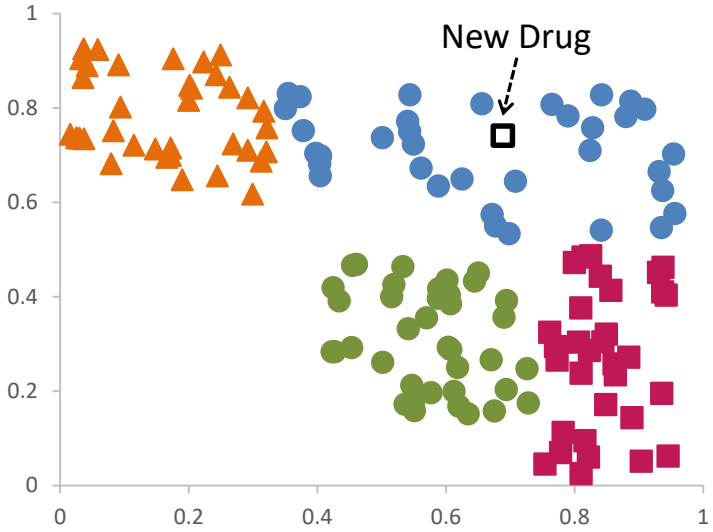
Mechanistic data allows industry to learn from failure, cutting time and costs of drug development

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

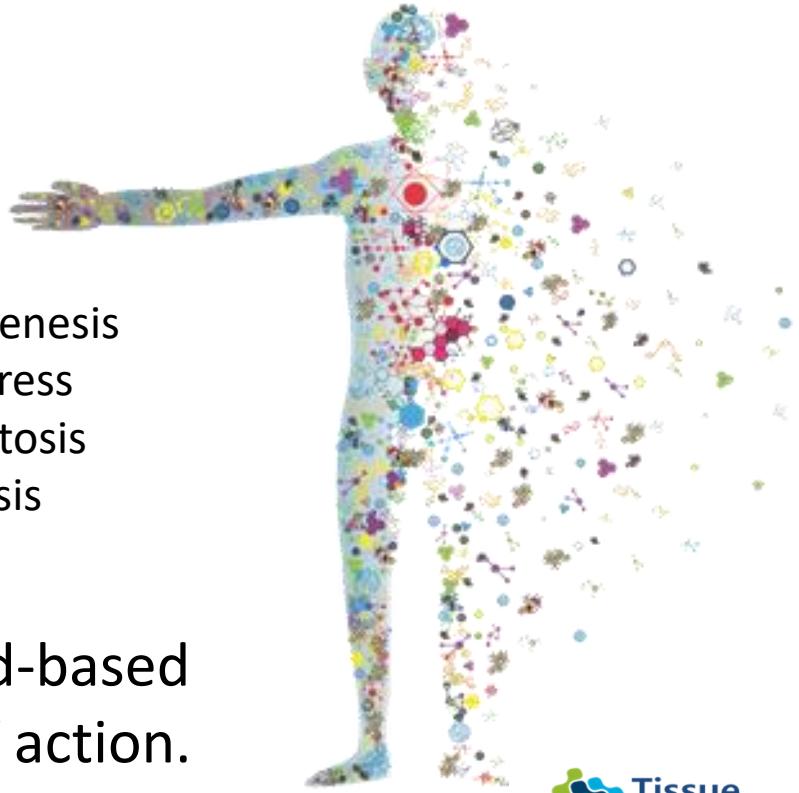
1. European Commission, SME Seal of Excellence (Feb. 2019)

Tissue Dynamics

Cloud-Based Metabolic Fingerprinting



- Lipogenesis
- ER-Stress
- Apoptosis
- Fibrosis



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

Tissue Dynamics

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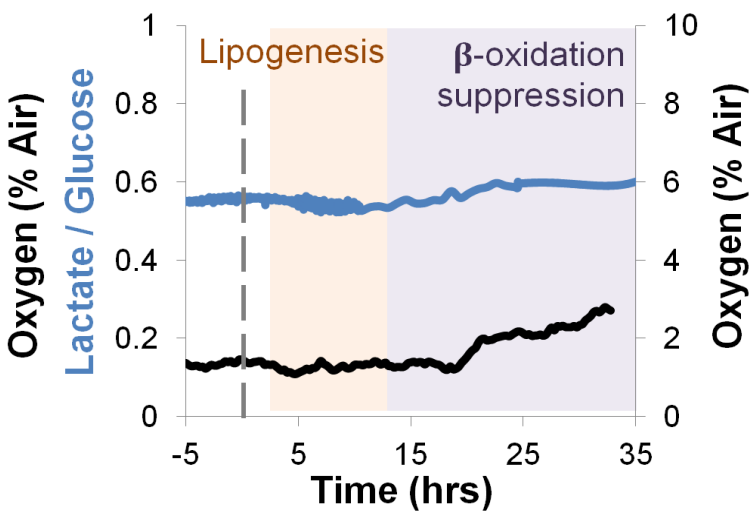
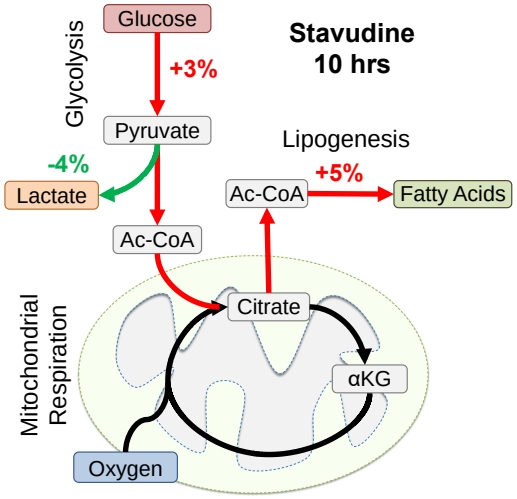
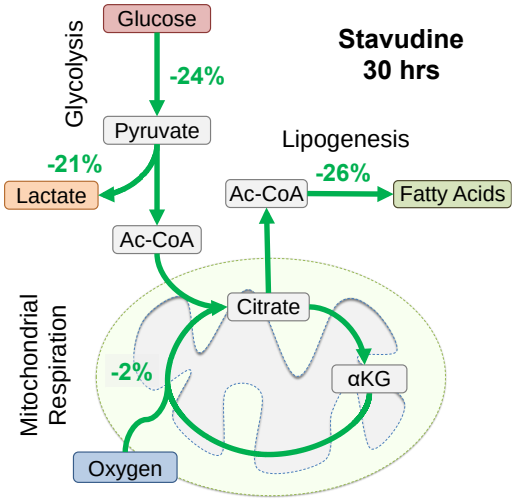
Stavudine Metabolic Analysis







- 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**