Functional coupling of human microphysiological systems

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Today’s questions

- What is the biggest problem we are addressing?
- In testing drugs and toxins, where are we today?
- What are microphysiological systems?
- How do you keep MPS systems alive?
- How do you couple MPS organs together?
- Do we need recirculation?
- What can we measure from MPS models?
- How can we control an MPS system?
What is the biggest problem we are addressing?
Biology spans lots of space and time

But it more complicated than that…

- Genomes
- Molecules
- Networks
- Cells
- Organs
- Animals

Images by CFDRC and VUMC
Biology has complex multiscale interactions

- Homeostasis is an orbit around an attractor in $10^4$ to $10^6$ dimensional phase space.
- Aging and disease are extended trajectories in that space.
- Drugs can act at any scale.
- Animals are not perfect models of humans.
John Wikswo’s goal – Determine how best to fit two new *Homunculi* species into the biomedical research ecosystem.

*Homo chippiens* NanoHuman (nHu)

*Homo minutus* MicroHuman (µHu)

Organ-on-chip tox-safety studies can use either single organs or a coupled-organ homunculus

The “Media Volume” problem

Conventional culture
- A typical picoliter cell requires a nanoliter of media per day.
- A 10 μm layer of cells is covered by a 10,000 μm layer of media.
- 1 fluid change/day
- Metabolites, endocrine, autocrine, and paracrine factors are diluted 1000-fold.

Microfluidic tissue culture
- A typical picoliter cell requires a nanoliter of media per day.
- A 10 μm layer of cells is covered by a 2 μm layer of media.
- 5000 fluid changes/day
- Metabolites, autocrine, paracrine, and endocrine factors are diluted by only 1.2x

Relative sphere sizes: nL media vs pL cell
In testing drugs and toxins, where are we today?
How do you test drug efficacy and toxicity?

- **Cells in vitro**
  2D biology on plastic: Many biological experiments are conducted on cells that
  - have cancer,
  - are inbred,
  - are diabetic,
  - are potatoes on a stiff plastic couch without exercise,
  - enjoy neither gender nor sex,
  - live almost entirely in the dark,
  - gorge themselves on sugar once a day,
  - may be slowly suffocating in an increasingly acidic environment,
  - live in their own excrement,
  - never bury their dead,
  - may take a complete or only partial bath every day or two,
  - and talk only to cells of like mind.

One might get reproducible, statistically significant results, but are they relevant to human biology and disease?

- **Animals**
  Animals, including non-human primates, are not people and have significant genetic and physiological differences.

- **People**
  The worst possible toxicity test on a human is the one that you did not intend to conduct…
What are microphysiological systems?
What do organs-on-chips look like?

Perforated PDMS membranes support pulmonary endothelial and epithelial cell layers (Ingber group, Wyss, Harvard)

Interfaces are important, and endothelia can protect cells.

Huh et al., Science, 2010
Lung-on-a-Chip

Liver-on-a-Chip

https://emulatebio.com/

https://thenewstack.io/organs-on-chips-emulates-human-organs-for-better-biomedical-testing/
Microvascular nanoparticle delivery assay

Schematic of the BBB Model. Apical chamber (outer channels) are for culture of vascular (endothelial cells) while basolateral chamber (central chamber) are for culture of brain tissue cells (astrocytes, pericytes, neurons). Porous architecture enables communication between the vascular and tissue cells.

Create a realistic 3D co-culture with real time monitoring of cell-cell interactions between tumor, stromal, vascular and immune cells.
Mammary gland on a chip
Lisa McCawley and Dmitry Markov, Vanderbilt

T cells in a lymph node on a chip
Shannon Faley, Kevin Seale and John Wikswo, Vanderbilt

Brain on a chip
Jacquelyn Brown and John Wikswo, Vanderbilt

Heart on a chip
Veniambin Sidorov and John Wikswo, Vanderbilt
Organ Chip Commonalities

• The organ chips are in a variety of flavors
  – Stretched 2D interfaces
  – Static 2D interfaces
  – Perfused 2D interfaces with 3D matrix on one side
  – Open transwells
  – Closed channels
  – Vasculature on the walls of collagen channels
  – Unattached cells in traps.

• Many groups are trying to create a 2D, 2D+, or 3D architecture with perfusion to more accurately recapitulate the *in vivo* environment.

• MPS developers, NIH, DoD, FDA and EPA want to encourage the use of these technologies by Pharma, environmental toxicologists, and basic scientists.
Brain Organoids as Developmental Models

How do you test drug efficacy and toxicity?

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• **3D Organoids**
  
  Complex 3D biology is a better model than 2D biology.
  Are self-organizing models with tissue-level functions and disease phenotypes.
  Demonstrate development.
  Can be transplanted.
  Medium throughput
  May require engineered hydrogels
  Hard to perfuse
  Individual organoids are hard to replicate
  No barrier functions
  Hard to visualize when living
  Hard to integrate with other organ systems

Contributions from Kapil Bharti (NIH/NEI)
How do you test drug efficacy and toxicity?

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- **Organ Chips**
  - Better than 2D biology ideal for barrier functions
  - Can reproduce physiological flows
  - Can use minimal media volumes
  - May ultimately reduce drug costs
  - Possible to build a single-patient homunculus
  - Could build animals-on-chips

- **3D Organoids**
  - Complex 3D biology
  - Self-organizing models with tissue-level functions and disease phenotypes
  - Demonstrate development
  - Can be transplanted
  - May require engineered hydrogels
  - Hard to perfuse
  - Individual organoids are hard to replicate
  - No barrier functions
  - Hard to visualize when living
  - Hard to integrate with other organ systems
  - Not fully validated vs in vivo, e.g., no WGCNA yet
  - Human cells are expensive and can be needed in quantity can’t be transplanted

Contributions from Kapil Bharti (NIH/NEI)
MicroPhysiological Systems (MPS) include organs-on-chips, tissue-chips, 3D transwells, and [self-assembling and engineered] organoids.

Matt Wagoner, AstraZeneca, 2015
Test drugs in homunculi!

We are building homunculi because
- Human biology is complex
- Homunculi can simplify:
  - Drug development
  - Environmental toxicology
  - Physiology
  - Understanding disease

How do you build homunculi?
- Use human cells to make microfluidic organ chips that work like the real organs.
- Connect organs together.
- Do lots of things at the same time.

Unexpected human organ-organ interaction. No human dies.
Where do MPS models belong?

- Improve the transition from cells to animals to humans?
- Introduce human cells and tissue-equivalences earlier?
- Explain mechanism of action when questions arise?

“Clinical trials in a dish have potential application across multiple areas of drug development – from early drug discovery, to later lead optimization for safety and efficacy testing, to regulatory safety assessment.

“If properly validated, there is the potential for clinical trials in a dish to replace animal testing, some types of clinical trials, or to be used to select patient populations or even individual patients most likely to benefit or least likely to be harmed by therapies.

“Some of these potential applications are farther from reality, while others are relatively close to implementation.”
The Grand, Organ-on-a-Chip Vision for Drug Development

Imagine instead of animals ....

• A “human-on-a-chip” ...

• ... using cells from patients who are in the hospital today

• ... and your Human-on-a-Chip helps you understand whether that person will have a pharmacologic response to your drug

• ... whether that drug is in Phase 3, or Phase 1, or was just this morning synthesized by a medicinal chemist for a novel drug target that may, or may not, bring tremendous value to one or more patients

• ... and by the way, you can also predict the absorption, disposition, metabolism, drug-drug interactions, and safety risks for this drug in the intended patient, as well as a panel of 100’s of other patients with that disease.

• And if something goes wrong, you learn this before patients are put at risk.  

Dave Watson, 2017
How do you keep MPS systems alive?
Single Organ Topologies

\[ R_A \quad \text{Organ} \quad R_V \]  
\[ R_{ECF} \quad \text{Organ} \quad R_{Lymph} \]

FE Block III, FE Block Jr., JP Wikswo, in preparation
Syringe pumps in an Incubator

Advantages
• Commercially proven
• In common use

Disadvantages
• Large footprint
• Large syringe volumes
• Expensive
• Need dual-pump and valve for long-term continuous perfusion
• Hard to recirculate
Pressurized reservoirs in an incubator

**Advantages**
- Compact
- Pressure control of flow
- Low cost
- Low risk
- First to market
- Easily Emulated

**Disadvantages**
- Hard to recirculate
- Difficult to run two or more interconnected organs
- Lack of valves reduces logical options
Rotary planar peristaltic micropump (RPPM)

Balls are driven in a circle over microfluidic channels by a rotating disk of PDMS while being held a plastic cage.

Imagine rolling an orange in a circle between your hands.

RPPM with dye-filled channels

Four different sizes so far. Pumps can operate > 2.5 million cycles.

Arduino controller for four RPPMs

Gould, Huang, et al., PCT/US2011/055432

Parker Gould, Loi Hoang, et al., in preparation
Long-term mammosphere culture

Thick Tissue Bioreactor (TTB)

• Advantages
  – Compact
  – Low cost
  – Confocal compatible
  – Gravity or pumped flow
  – Multichannel
  – Fast to market
  – Well suited for single-organ studies

• Disadvantages
  – Not yet reduced to mass production design
  – A bit too simple for organ-organ studies

MCF10-CA1d 21-days in bioreactor

MCF10-CA1d dose-response to Docetaxel

Markov et al., LoC, 2012
**Challenge:** Syringe pumps are expensive and not easy to move during handling.

**Solution:** Our pumps and valves allow for stand-alone IOMs at a low cost.

*Normally closed rotary planar valves (NC-RPV)* allow us to control perfusion, drug delivery, and sampling on-chip.

United States Patent, 9,618,129 B2

**Rotary Planar Peristaltic Micro-pumps** and **Rotary Planar Valves** enable a Perfusion Controller for a Neurovascular Unit on a Chip.
Perfusion controller with recirculation

Automated control with three valves and one pump:
- Cell loading
- Perfusion
- Drug delivery
- Recirculation
- Sampling

Mode 1.2
Inject Drug only into Organ N

One-pole, four-throw valve

Two-pole, three-throw valve

1 pole, 4-throw valve

On-chip pump

Organ N Effluent Bus

On-Chip Pressure Sensors

Waste

µClin Analyzer

Output

Wikswo
11/28/2011
VIIBRE’s organ module concept

- Create general purpose components
  - Pumps, valves, baseplates, bubble traps, microcontroller, software…
  - Assemble components into modules
    - Perfusion Controller, MicroClinical Analyzer, MicroFormulator, InterConnect…

- Each organ operates as an individual module
  - Low-volume on-board pumps and valves
  - Perfusion, oxygenation, waste removal..
  - Recirculation optimizes media conditioning
  - Replace media at a physiological rate
  - Fluidics disposable after use, hardware reusable

- The organ modules can be coupled together
  - Passive tubing (1 cm of 360 µm PEEK tubing = 20 nL/cm)
  - Can include active valves as required (load, recirculate, sample…)
  - Cardio-pulmonary assist

- System sensing and closed-loop control
  - Mechanical, electrical, chemical, optical
  - Real-time electrochemical metabolic sensing

- Missing organ MicroFormulator

- Untargeted, in-line, near-real-time analytics
How do you couple MPS organs together?
Coupled organs support PKPD

- Coupled organs on a single chip can scale either volumes or media exposure times
- Supports PKPD analyses

Nature 471, 661–665 (31 March 2011)

Pump connectors and tubes

Lids
Cell culture compartment optional with cell culture inserts 96 well (e.g. Transwell®, Millicell®) or Ø8mm MatTek Inserts®

Adapterplates

PDMS layer (microscopic slide format)

Glass slide

Support (temperature control: optional)

View from below:

Microfluidic channels
Urine circuit
blood circuit

on-chip micro-pump
culture compartments

https://www.tissuse.com
The “Volume problem”

A) A pL cell requires a nL of fresh media each day.

B) The “Volume problem” in dishes and wells: paracrine, autocrine and endocrine factors diluted by a factor of 1000.

C) Microfluidics can reduce the volume of a single organ-on-chip.

D) Pressurized or piipetted reservoirs may not solve the volume problem

E) Integrated microfluidics should solve the coupled organ volume problem.
NIH-NCATS MPS Integration

Arterial System

- Neurovascular Unit
- Intestine
- Liver
- Kidney
- Muscle

Physiology Sense and Control: Mech, Elec, Chem, Optical

Cardiopulmonary Assist

Pressure, O₂, CO₂, pH, Osm Sense and Control

Missing Endocrine \( \mu \)Formulator

Venous System

- Neurovascular Unit
- Intestine
- Liver
- Kidney
- Muscle

ME-UF

Vanderbilt

Hopkins, Baylor

Pittsburgh

Washington

Duke
Serial transport of fluid between individual organs, even across state lines
Work Flow for Functional Coupling Experiment

Goal: Couple Gut, Liver, Brain, and Kidney

Vernetti, et al., Scientific Reports, 2017
Work Flow for Functional Coupling Experiment

**Vitamin D3 Transport and Metabolism**
- **Intestine**
  - Uptake & transport
- **Liver**
  - Cytochrome P450 hydroxylation
    - 25 (OH) Vitamin D3
  - 25 (OH) Vitamin D3-DBP
- **Kidney**
  - Cytochrome P450 hydroxylation
    - 1α,25(OH)₂ Vitamin D3
    - 24,25(OH)₂ Vitamin D3
- **Blood Brain Barrier**
  - Penetration of all forms

**Terfenadine Transport and Metabolism**
- **Intestine**
  - Uptake & transport
  - Cyp3A4 metabolism, P-glycoprotein transport
- **Liver**
  - Low Fexofenadine clearance
- **Blood Brain Barrier**
  - Penetration of all forms

**TMA Transport and Metabolism**
- **Intestine**
  - TMA microbiome product
  - Uptake & transport
- **Liver**
  - TMA conversion to TMAO*
- **TMA, TMAO**

**Key Concordances Between MPS and Clinical Fate for Three Test Agents.**
Key: Uptake - by jejunum endothelial cells; Transport - from apical to basolateral media; → = Metabolism; CounterTrans = Transport from basolateral to apical media; est. = estimated. Excreted - into proximal tubule lumen; LOQ = limit of quantitation; Penetration - through blood-brain barrier.

<table>
<thead>
<tr>
<th>Test Agent/Metabolites</th>
<th>Clinical MPS Model</th>
<th>Intestine</th>
<th>Liver</th>
<th>Kidney</th>
<th>BBB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TMA TMAO</strong></td>
<td>Clinical</td>
<td>Uptake &amp; Transport</td>
<td>TMA → TMAO &lt; 5% TMA Clearance</td>
<td>&gt; 95% TMAO Excreted</td>
<td>TMAO Penetration: Unknown</td>
</tr>
<tr>
<td><strong>TMA</strong></td>
<td>MPS</td>
<td>Uptake &amp; Transport</td>
<td>TMA → TMAO &lt; 1% TMA Clearance</td>
<td>~46% TMAO Excreted</td>
<td>26% TMAO Penetration</td>
</tr>
<tr>
<td><strong>Terfenadine (Ter)</strong></td>
<td>Fexofenadine</td>
<td>Ter → Fox; Fox CounterTrans</td>
<td>&lt; 1% Bio T &gt; 95% Fox Clearance</td>
<td>11% Fox Excreted</td>
<td>~0% Fox Penetration</td>
</tr>
<tr>
<td><strong>MPS</strong></td>
<td>Ter → Fox; Fox CounterTrans</td>
<td>&lt; 1.4% Bio T (est.) &lt; 80% Fox Clearance</td>
<td>~ 1% Fox Excreted</td>
<td>~ 0% Fox Penetration</td>
<td></td>
</tr>
<tr>
<td><strong>Vitamin D3 (VD3)</strong></td>
<td>Clinical</td>
<td>Uptake &amp; Transport</td>
<td>No metabolism</td>
<td>VD3 → 25(OH)VD3</td>
<td>25(OH) VD3 → 1α,25(OH)₂VD3 &amp; 24,25(OH)₂VD3</td>
</tr>
<tr>
<td><strong>MPS</strong></td>
<td>Uptake &amp; Transport</td>
<td>No metabolism</td>
<td>VD3 → 25(OH)VD3 &amp; 24,25(OH)₂VD3</td>
<td>1α,25(OH)₂VD3 &amp; 24,25(OH)₂VD3 below LOQ</td>
<td>0.4% VD3 &amp; 6% 25(OH) VD3 Penetration</td>
</tr>
</tbody>
</table>

Vernetti, et al., Scientific Reports, 2017
Do we need recirculation?
Basic Perfusion Controller

Bubble & Debris Trap

Pump

Organ-on-Chip

$R_{OoC}$
To recirculate or not recirculate?

Advantages
• Animals recirculate
• Reduces media volume
• Physiologically realistic
• May allow studies of metastasis and infection
  – Best done with endothelial barriers
• Media addition/removal maintains concentrations

Disadvantages
• Technically harder
• Must trap cell debris
• Balance local reservoirs with a global reservoir
• May require kidney and liver to process media
• May require closed-loop control
• Need to allow for gradual media exchange
- The Organ-on-Chip is perfused by a circulating pump (upper) that exchanges media with a closed reservoir.
- The MicroClinical Analyzer is used to measure the glucose, pH, and lactate as the media is withdrawn from the closed reservoir and replaced with fresh media by a second pump (lower).
- The rate at which media has to be replaced can be determined for different volumes of recirculating media.

Mark Fauver and John Wikswo
Organ Recirculation and Organ-Organ Interconnects

Local Organ Recirculation
- Flow rate
- Local reservoir volume
  - Could be reduced with time
- Fluid replacement rate
  - Could increase with time

Global Mixing
- Time-division multiplexing of fluid exchange to each organ
  - Compensate for organ size differences
- Global fluid replacement rate
  - Media to replace withdrawal to waste or sampling
  - H₂O to replace evaporation

Volumes
- Organ
  - Cells
  - Vascular fluidic system & reservoir
  - Bubble trap and debris filter
  - Interstitial space
  - Interstitial fluidic system
  - Interstitial reservoir
- Central
  - Central reservoir
  - Recirculation fluidics

Separate control of organ recirculation and interconnection!
What can we measure from MPS models?
Monitoring organ health/toxin response

- Cellular morphology
  - Requires fluorescence microscopy
  - Organ-on-chip module should be HCS confocal compatible
- Genetically encoded fluorescent reporters
- TEER
- Bioenergetics
- Protein production
  - Albumin
  - Bile acids
  - Cytokines
  - Cyp activity
  - LDH release
- Transcriptomics
- Drug metabolism
- Untargeted metabolomics
- Metabolic activity (glucose, lactate, pH, oxygen)

The sensitivity of many assays is set by the ratio of cell volume to media volume!
ViIBRE Analytics for Organs on Chips

• Organs-on-Chips
  – Brain (in regular production and testing drugs)
  – Mammary Gland (demonstrated, published, moving towards production)
  – Cardiac Muscle 3D construct (demonstrated, published, being refined)
  – Fetal Membrane (under development by Osteen @ VU)
  – Gut (In prep with Donowitz @ JHU and Estes @ Baylor; Rericha and Lau @ VU)
  – Developmental bone-joint (In prep with Tuan @ U. Pitt)

• End Point Evaluations
  – Myocardial elastomechanics
  – TEER – transendothelial electrical resistance (real-time)
  – Barrier active transport (off-line microplate or LC-MS)
  – Barrier permeability (FITC dextran diffusion)
  – Cytokines (ELISA)
  – Fluorescence imaging
    • Cell survival – live/dead assay
    • Mitochondrial membrane potential
    • Transmembrane potential
  – Metabolic activity (real-time glucose, lactate, pH, oxygen)
  – Cell morphology
  – Confocal 3D reconstruction
  – Proteomics and metabolomics (ion mobility-mass spectrometry)

The sensitivity of many assays is set by the ratio of cell volume to media volume!
Sample preparation

metabolites extracted using ice cooled methanol:H₂O (80:20), incubated -80°C overnight, spun down at 15,000 rpm, 15 min dried down in vacuo

Sample Acquisition

LC IM-MS/MS of metabolite extracts

LPS or Cytokine treated samples

Data Alignment and Biostatistical Analysis

Progenesis QI

Pathways Analysis

<table>
<thead>
<tr>
<th>Pathways Analysis</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Vitamin E metabolism</td>
<td>8.00E-05</td>
</tr>
<tr>
<td>Glutathione Metabolism</td>
<td>1.13E-03</td>
</tr>
<tr>
<td>Prostaglandin formation from arachidonate</td>
<td>6.48E-03</td>
</tr>
<tr>
<td>Aspartate and asparagine metabolism</td>
<td>9.95E-03</td>
</tr>
<tr>
<td>Drug metabolism - cytochrome P450</td>
<td>9.97E-03</td>
</tr>
</tbody>
</table>

Network and Pathway analysis

Mummichog

Brown et al., J. Neuroinflammation, 2016
Neuroelectric recordings: hiPSC neurons

MFR plots

Temporal Spike Raster Plots

Electrode Cluster Baseline

D-AP5 (80 µM) NMDA-receptor antagonist

NBQX (50 µM) AMPA-receptor antagonist

Tetrodotoxin (TTX, 1 µM) Inhibitor of Na⁺ channels

Recovery

3Brain 4096 multielectrode array can measure hiPSC neuron electrical activity and drug response

Aaron Bowman and Diana Neely
How can we control an MPS system?
Multi-MicroFormulators for testing the effects of drug timing

- Matt Wagoner – “Your µF is great, but I need 96 channels.”

- What can you learn by lengthening or shortening the effective PK profile of a drug in vitro?

- What is the optimal timing for repeated or multi-drug dosing?

Perturbing Cellular Pathways

- Currently performing additional experiments to understand signaling dynamics and its influence on cell fate

Funded by AstraZeneca.
In operation at AZ - Waltham, MA since January, 2016

Funded by AstraZeneca and NIH-NCATS
Diseases and optimal drug dosing are circadian.
Diurnal variations of liver-regulating hormones

Cyr, Avaldi, and Wikswo, Experimental Biology and Medicine, 2017
Which endocrine organs / hormones do we need?

Cyr, Avaldi, and Wikswo, Experimental Biology and Medicine, 2017
Diurnal variations of organ-regulating hormones

- Neurovascular Unit
- Kidney
- Muscle
- Adipose
- Heart / Cardiovascular

Cyr, Avaldi, and Wikswo, Experimental Biology and Medicine, 2017
Diurnal variations of organ-regulating hormones

Cyr, Avaldi, and Wikswo, Experimental Biology and Medicine, 2017
Organ-on-Chip Technical Challenges

- What is the size of each organ?
  - Scaling criteria
  - Creation and maintenance of cellular heterogeneity
  - Scaling will fail at the single-cell level
- How do you control fluids within the volume and cost budgets?
  - 4.5 mL for milliHuman, 4.5 μL for a microHuman
  - Minimize pump, tubing and interconnect dead volume
  - Fluid makeup after sample withdrawal
  - Eliminate bubbles
  - Need thousands of units operating for a month
- Analytical chemistry in nL bioreactors
  - Electrochemical sensing of pH, glucose, lactate, oxygen
  - Optical monitoring of [Ca^{2+}]_{in}
  - UPLC/MALDI/nESI ETD IM-MS/MS Omni-Omics
  - Non-specific analyte binding
  - Integration, mining, and interpretation of Omni-Omic data
- Blood surrogate
  - Universal media without serum
  - Transport protein
  - Osmolarity
  - Perfluorocarbon or hemoglobin O_{2} carrier
- Putting organs together and controlling each and all of them
  - Scaling laws revisited
  - Delivering oxygen without excess fluid
  - Controlling metabolic activity
  - Maintaining correct salinity
  - Preventing, controlling or utilizing oscillations
  - Utilizing Fisher randomized multiparametric questionnaires
- Accounting for missing organs
  - Adding missing compounds
  - Removing compounds that would be metabolized by missing organs
- Modeling of coupled organ systems
  - Multiphysics to design
  - PK/PD of drugs in multi-organ systems
  - Inverse models for data interpretation
  - Learning from regulatory noise
- How do we diagnose health vs disease?
- What will a milli/microHuman cost?
- Utilizing organs on a chip
- How accurate a mHu or μHu can we produce? Need to produce?

Wikso et al., IEEE Transactions on Biomedical Engineering, 2013
Immediate Applications for MPS models

• Disease Biology / Pharmacology
  – Discovery of novel mechanisms of human diseases
  – Identification of novel compounds including probes, leads, clinical candidates
  – Discovery of the mechanism of action of drug candidates
    • On target
    • Off target

• ADME-PK-Clinical Pharmacology
  – Early identification of problematic human haplotypes & drug–drug interactions (DDIs) for small molecules
  – Improved prediction of human exposure for compounds and clinical formulations

• Toxicology
  – Earlier termination of toxic drugs
  – Avoid inappropriate drug terminations

Dave Watson, Rosemarie Hunziker, and John Wikswo, Experimental Biology and Medicine, 2017
In Vitro Problems that may need today’s MPS capabilities

- Access to both sides of barriers polarized by shear flow
  - Blood-brain barrier
  - Blood-testis barrier
  - GI tract
  - Angiogenesis / vasculogenesis

- Mechanically active systems
  - Alveolar interface
  - Gut
  - Skeletal, smooth, and cardiac muscle
  - Developmental bone-joint

- Complex, well-defined heterogeneous 3D cultures
  - Liver
  - Brain
  - Skin
  - ...

- Coupled organs for drug-drug interactions and ADME-Tox
  - Gut-liver
  - Liver-brain
  - Gut-kidney-liver
Problems that are at the MPS cutting edge

• The full metastatic cascade
  – Localized formation of the primary tumor
  – Intravasation into vascular and lymph systems
  – Dissemination through vascular and lymph systems
  – Extravasation into competent organ
  – Colonization and proliferation with seed-soil interactions

• Testing immuno-oncology drugs
  – Requires isogenetic innate and adaptive immune system, tumor, and metastatic niche to avoid host-versus-graft reactions and MHC-HLA incompatibilities.
  – May require organ-specific lymph nodes, immune-active spleen and bone marrow for proper programming of multiple types of immune cells.
  – CD34+ progenitor cells and B cells have yet to be derived from iPSCs.
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