Advancing New Alternative Methods at FDA

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MPS World Summit 2023

Disclaimer: *This presentation reflects the views of the authors and does not necessarily reflect those of the U.S. Food and Drug Administration. Any mention of commercial products is for clarification only and is not intended as approval, endorsement, or recommendation.*

Outline

- FDA's Alternative Methods Working Group
  - Accomplishments
- Food and Drug Omnibus Reform Act of 2022 (FDORA)
- Center Activities
  - CDER
  - CBER
  - CFSAN
  - CVM
  - NCTR

Alternative Methods Working Group (AMWG)

Office of Chief Scientist (OCS), Office of Commissioner

- Chaired by Drs. Fitzpatrick (CFSAN) and Mendrick (NCTR), includes members from each Center, Office of Regulatory Affairs, and OCS

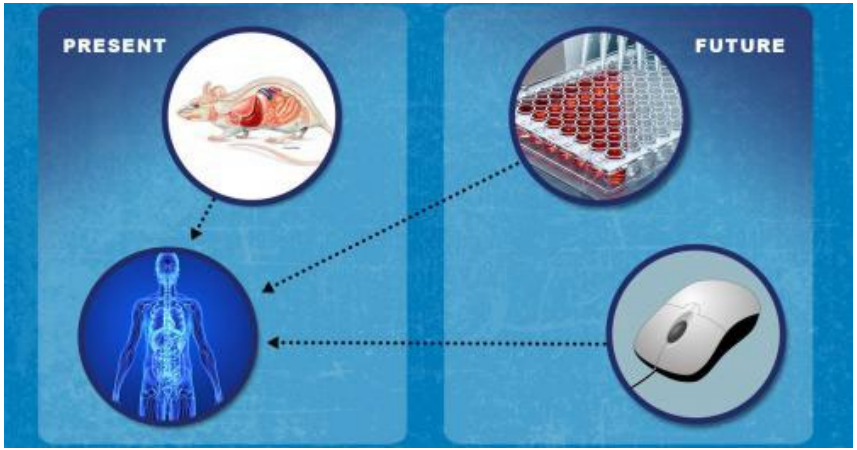
Leadership Council: researchers and regulators

Kevin Ford (CDER)
Nakissa Sadrieh (CDER)
Paul Brown (CDER)
Claudia Wrzesinski (CBER)
Kyung Sung (CBER)
Peter Goering (CDRH)
Shelby Skoog (CDRH)
Kristi Muldoon Jacobs (CFSAN)
Suzy Fitzpatrick (CFSAN)

Luis Valerio (CTP)
Barry Hooberman (CVM)
Dayton Petibone (NCTR)
Donna Mendrick (NCTR)
Paul Howard (ORA)
Brianna Skinner (OCS)
Tracy Chen (OCS)
Tracy MacGill (OCS)

Report available on the FDA webpage

Advancing Alternative Methods at FDA



- Website for alternatives at FDA (https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda)
- Inviting developers to present their technologies
- Posting FDA-authored peer-reviewed publications and presentations

Transparency

Contact information: alternatives@fda.hhs.gov

AMWG Accomplishments

- Educational seminars (1-2 x per month) by internal researchers, external users/researchers, platform providers, etc.
- Increased cross-center interactions and collaborations with outside parties
- The FDA's fiscal year (FY) 2023 Budget includes $5.0 million in new funding to implement an FDA-wide New Alternative Methods Program
  - Spur the adoption of NAMs for regulatory use that addresses the 3Rs and improves predictivity of nonclinical testing
- Paper in development

Outline of Paper

- History at FDA in promoting MPS
- Why do we need animal MPS?
- In what areas are they needed and what species and tissue types should be prioritized?
- Animal MPS development

Historical Perspective

- DARPA and NIH funded the generation of human but not animal MPS
- Almost all centers at FDA are working with commercial and/or in house versions of human MPS
- CFSAN is working with NCATS on developing a human NMJ chip
- FDA's MCMi has partnered with NIH to fund work developing MPS models of viral infection and pathogenicity such as the development of NHP MPS models to bridge nonclinical and clinical data
- FDA and NASEM convened a workshop in January 2021 on "Microphysiological Systems (MPS): Bridging Human and Animal Research"

Why Animal MPS?

Gain confidence in human MPS

- There needs to be more confidence in the performance and predictive capabilities of such systems to use for regulatory decision-making
- One way to fill this gap is understanding how animal MPS compares with animal *in vivo* data for toxicity and efficacy
- This need crosses multiple FDA Centers
- The need to complete the rat *in vitro/in vivo* and human *in vitro/in vivo* parallelogram has been discussed at the MPS Berlin workshop in 2019 and the FDA-IQ MPS Affiliate workshop and reflected in their resulting publications

Marx et al., ALTEX 2020;37(3):365-394; Baran et al., 2022;29(2): 297-314

Areas of Need and Species

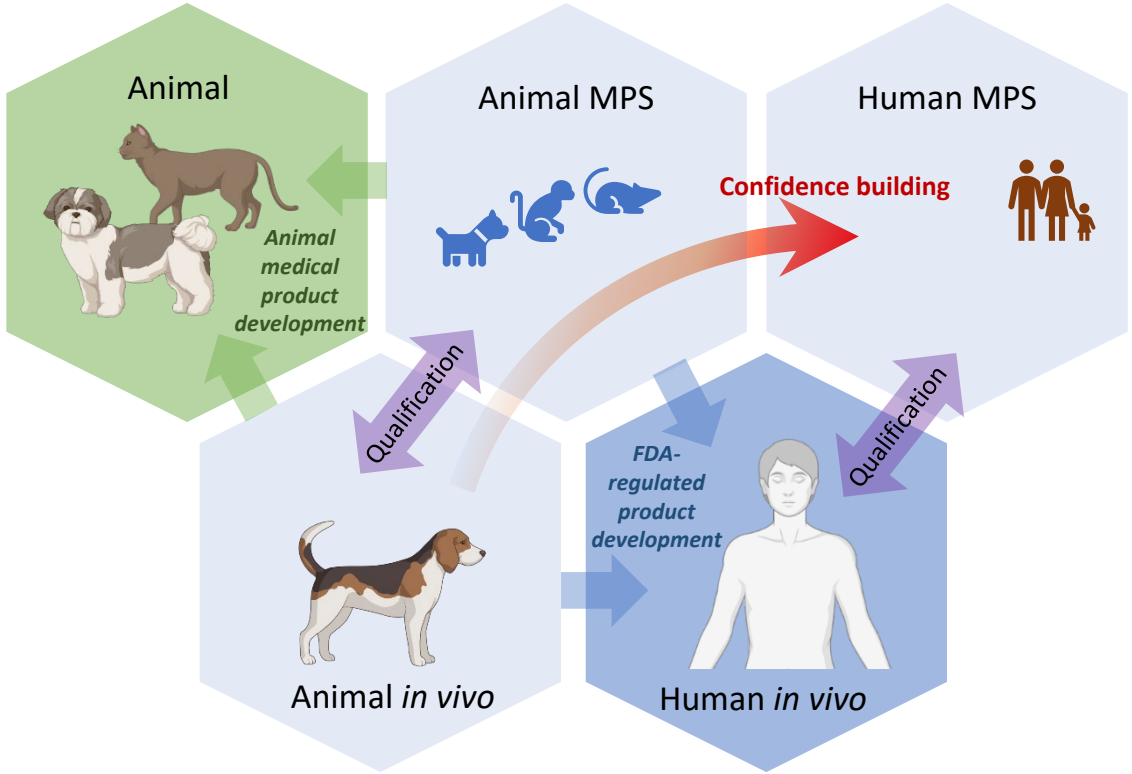
- Animal drugs
  - 7 major animal species and multiple breeds (e.g., 195 breeds of dogs)
  - Interest in studying canine responses at population level and within a species
- One Health
  - ~ 75% of emerging infectious diseases are zoonotic in nature (e.g., SARS-Co-2)
  - Need human and animal (e.g., bat, canine) chips

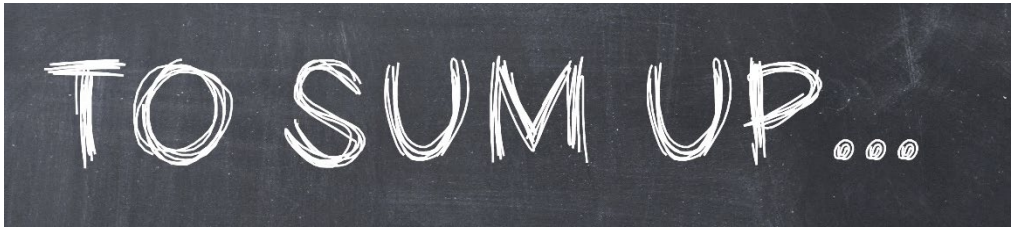
Areas of Need and Species

- CDER
  - CDER has a need for multispecies liver MPS to better assess the value or need for *in vivo* animal studies, as well as to complement any human tissue-based liver MPS
  - CDER and NCTR are developing a rat MPS liver model and hope to move to other species depending on funding

Areas of Need and Species

- CFSAN and CDER
  - Interested in animal and human MPS barrier models of gut and skin
- FDA centers
  - Broad interest in animal MPS that could be used for carcinogenicity assessments, countermeasures, reproductive studies bridging between *in vivo* animal and human MPS


Animal
Animal MPS
Human MPS
Confidence building
Animal medical product development
Qualification
Qualification
FDA-regulated product development
Animal in vivo
Human in vivo



- FDA is a diverse organization whose regulatory authority ranges from cosmetics to foods to biologics to drugs to devices

- How can FDA promote the development of these models?

- One funding source: Omnibus bill directs NIH's SBIR programs to provide funding for "Organotypic models using cells from rat or mouse models or other experimental animal models, with a focus on comparisons between *in vivo* and *in vitro* toxicity endpoints"

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Food and Drug Omnibus Reform Act of 2022 (FDORA)

- Replaced “preclinical tests (including tests on animals)” with “nonclinical tests”
- Defined nonclinical tests as: “a test conducted *in vitro*, *in silico*, or *in chemico*, or a nonhuman *in vivo* test, that occurs before or during the clinical trial phase of the investigation of the safety and effecti of a drug. Such tests may include the following:

  (1) Cell-based assays.

  (2) Organ chips and microphysiological systems.

  (3) Computer modeling.

  (4) Other nonhuman or human biology-based test methods, such as bioprinting.

  (5) Animal tests.”

Purpose of Nonclinical Data: Regulation

- IND content and format [21 CFR 312.23]
  - Pharmacology and Toxicology Information [(a)(8)]
    - The regulation requires that the sponsor submit "Adequate information about pharmacological and toxicological studies of the drug involving laboratory animals or *in vitro*, on the basis of which the sponsor has concluded it is reasonably safe to conduct the proposed clinical investigations."
    - "Guidance documents are available from FDA that describe ways in which these requirements may be met."

Current Safety Testing Paradigm (CDER)

Key Issues Addressed by Pharmacology and Toxicology Studies Supporting Pharmaceutical Development

- Pharmacological effects and mechanism(s) of action
- Risk attributes of drug ADME
- Safe “first in human” starting dose
- Safe maximum exploratory doses in early clinical trials
- Possible consequences of chronic exposure
- Risks for special populations (e.g., pediatrics)
- Specific parameters to monitor more closely in clinical trials
- Risks that are difficult or unethical to assess in humans
- Mechanistic understanding of an adverse biological change observed in animals or humans

Animal Studies: “Required” vs. “Warranted” (Not Just Semantics)

- FDA does not “require” animal studies for assessing toxicologic risk, rather the state of the science dictates whether an animal study is “warranted” as being the most relevant to assessing risk.
- When the state of the science establishes that an alternative approach is equally capable of assessing risk, the FDA accepts the alternative approach.
  - Context of use dependent
    - *In vitro*: ocular irritation, skin sensitization, cardiovascular safety
    - *In silico*: genotoxicity of impurities
- ICH and FDA guidances all explicitly support development of alternative assays
- Unfortunately, there are no alternative methods that can replace repeat dose toxicity studies in animals at this time
  - Fully integrated physiologic system (metabolism, hormonal and immune responses, etc.)
  - Broad, tissue agnostic, assessment of toxicity

What FDORA Did and Didn't Do

- Didn't
  - Change the state of the science supporting the regulatory use of NAMs
  - Didn't remove a "requirement" for animal safety studies
    - There was no such requirement to remove
- Did
  - Provide greater clarity to stakeholders regarding the potential acceptability of these alternative sources of nonclinical safety information
    - May result in increased investment in developing and validating NAMs

Examples of Accepted Alternatives

- *Assessing the Credibility of Computational Modeling and Simulation in Medical Device Submissions* – This draft guidance describes risk-based framework that can be used in the credibility assessment of computational modeling and simulation (CM&S) used in medical device regulatory submissions
- *ICH S5(R3) Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals* – This guidance includes an annex devoted to alternative assays for the evaluation of malformations or embryo-fetal lethality. Specific alternative assays are not described, but the principles for determining when an assay is appropriately qualified to support the risk assessment of a drug product are explained
- *Oncology Pharmaceuticals: Reproductive Toxicity Testing and Labeling Recommendations Guidance for Industry* – This guidance discusses potential use of alternative assays, such as fit-for-purpose *in vitro* or *ex vivo*, or nonmammalian *in vivo* assays for assessment of reproductive toxicity

CDER Approach to NAMs, Part I

- In a 2020 paper, FDA/CDER provided a perspective on nonclinical testing strategies and briefly discussed the opportunities and challenges of using NAMs in drug development, especially for regulatory purposes

- A list of key needs were identified where the current approach is less than optimal and could benefit from supplementary or fit-for-purpose testing approaches, including NAMs

Avila, et al. *An FDA/CDER perspective on nonclinical testing strategies: Classical toxicology approaches and new approach methodologies (NAMs).* Regulatory Toxicology and Pharmacology, 114 (2020)

CDER Approach to NAMs, Part II

- A 2023 paper described the results of discussions with CDER review staff around current nonclinical testing strategies
- Multiple gaps and challenges in achieving the goals of nonclinical safety assessments with the current toxicology approaches were identified
- The goal was to provide potential topics for which specific Contexts of Use (COUs) could be developed for NAMs that might then refine current nonclinical testing strategies

Avila et al. *Gaps and challenges in nonclinical assessments of pharmaceuticals: An FDA/CDER perspective on considerations for development of new approach methodologies.* Regulatory Toxicology and Pharmacology, 139 (2023)

Example of CDER Identified Needs

General Toxicity

Goal of Nonclinical Safety Assessment	Key Needs (examples)
○ Determine safe "first-in- human" (FIH) starting doses and maximum doses in early clinical trials ○ Identify target organs of toxicity ○ Identify possible consequences of chronic exposure ○ Identify specific parameters to monitor more closely in clinical trials	○ Improve risk identification for rare and idiosyncratic toxicities, (e.g., DILI, immune-mediated, CNS-toxicity) ○ Identify species-specific toxicity ○ Correlate to *in vivo* plasma concentrations of investigational drug ○ Identify and qualify biomarkers that allow for noninvasive or less invasive clinical monitoring of toxicities that are not easily monitorable in subjects

(Adapted from Avila et al., 2023)

Alternative Method Qualification (CDER and CDRH)

- The first submission to the ISTAND Pilot Program was accepted in September 2022 for a tool that proposes to evaluate off-target protein binding for a variety of biotherapeutic modalities, potentially reducing or eliminating the need to conduct some of the more standard nonclinical toxicology tests

- CDRH established the Medical Device Development Tools (MDDT) program to qualify tools that medical device sponsors can choose to use in the development and evaluation of medical devices. These tools include “Nonclinical Assessment Models,” which are non-clinical test models or methods

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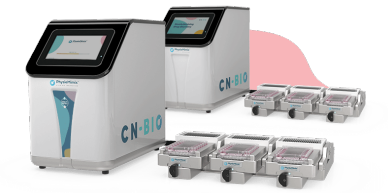
Research into MPS at CDER

- Evaluate commercially available MPS for the following criteria
  - Determine the criteria for a successfully cultured chip
  - Identify what endpoint assays can be measured
    - Define set of SOPs to assess the endpoints
  - Are the results reflective of tissue physiology?

- Publications help provide information for method developers relevant to:
  - Quality controls
  - Endpoint assays

*Clin Transl Sci.*2021;14:1659–1680

Collaborators



CN – BIO
- Airway
- Gut
- Liver

Emulate
- Airway

UC Berkeley
- Cardiac

Summary

- PI: Drs. Robert Geiger and Kevin Ford
- MPS currently being evaluated in CDER
  - Airway, Gut, Cardiac and Liver MPS
  - Future plans to connect gut and liver MPS
- Assessment of MPS
  - Identifying assays for characterization/standardization of chips
  - Identify the endpoint assays
    - Drug permeability, metabolism
  - Define SOPs

Type of MPS	**Drug study**
Airway chip	Drug permeability
Gut chip	Drug permeability/toxicity
Liver chip	DILI prediction
Cardiac chip	Cardiotoxicity

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CBER: Considerations for Biological Products

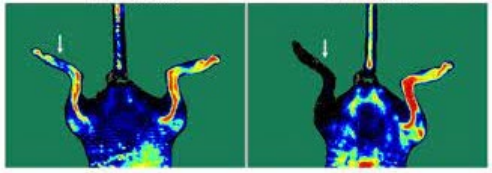
Dr. Kyung Sung

- Drugs are chemically synthesized and have a well-defined structure
- Biological products are complex and isolated from a variety of natural sources, including humans, animals, and microorganisms. Difficult to fully characterize by conventional testing methods
- Need to control manufacturing process and source material because biological products can be fragile, sensitive to manufacturing processes, available in limited amounts for testing and constrained inherent stability
- It is critical to develop new innovative test methods for biological product characterization that provide enhanced sensitivity, specificity, and predictive value
- Recent technological advancements have facilitated the development of biologics intended for a single or small number of patients (so-called individualized or bespoke therapies). It is critical to understand how novel and alternative methods could be used to characterize individualized therapies for the treatment of patients with rare diseases

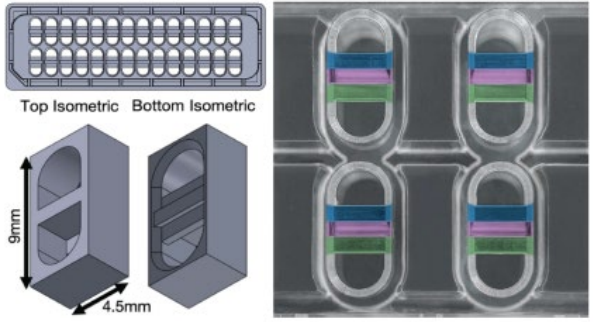
CBER: Human Blood Vessel Chips to Predict Vasculogenic Potency of Multipotent Stromal Cells

- Interest in using donated human multipotent stromal cells (MSCs) to treat patients with various vascular diseases. However, the ability of MSCs to stimulate vessel regeneration varies depending on the cell lines derived from donors and the manufacturing conditions under which the cells are prepared for therapeutic use
- CBER scientists use human blood vessel chips to identify MSCs that are more likely to stimulate blood vessel regeneration via paracrine interactions as an alternative to the traditional murine hindlimb ischemia model
- A quantitative method for predicting which MSCs will effectively stimulate vessel regeneration would improve manufacturers' ability to prepare safe and effective MSC products

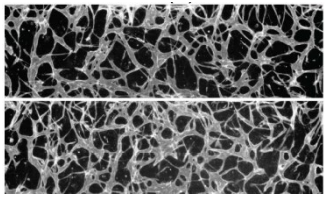
Traditional murine hindlimb ischemia model



High throughput human blood vessel chips enabling compartmentalized co-culture



Vasculatures formed within the chips



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CFSAN: NAMS Roadmap Plan for Botulinum Neurotoxin Assays In Regulatory Decision-Making

- Technical characterization and validation to qualify a neuromuscular junction (NMJ) chip for Botulinum Neurotoxin (BoNT) testing
- Replace animal testing in FDA's ORA network and public health laboratories for conducting foodborne botulism investigations and support regulatory reviews
- Advance the NAM as a predictive toxicological tool for risk assessment
- Support transferring the NAM and train FDA-wide laboratory scientists in technology as needed
- Support development of regulatory guidelines to meet CGMP practice in biopharmaceutical industries
- Advance the NAM as Drug Discovery Tool (DDT) for Botulinal Medical Countermeasure (BMC) development to treat botulism and other nerve agent poisoning

CFSAN and NCATS Partnership

- Collaborative framework to provide mutually beneficial logistical and operational support to manage and share expertise
- Provisions to protect or not disclose confidential, proprietary or trade secret nature, business or financial or research information, etc. as applicable
- Develop, Qualify, and Commercialize Tissue of Chip platforms as Drug Developmental Tool (DDT)

FDA Research Collaboration Agreement

Los Alamos NATIONAL LABORATORY

ananda™

In vitro solutions for *in vivo* results

Laval, QC, CANADA

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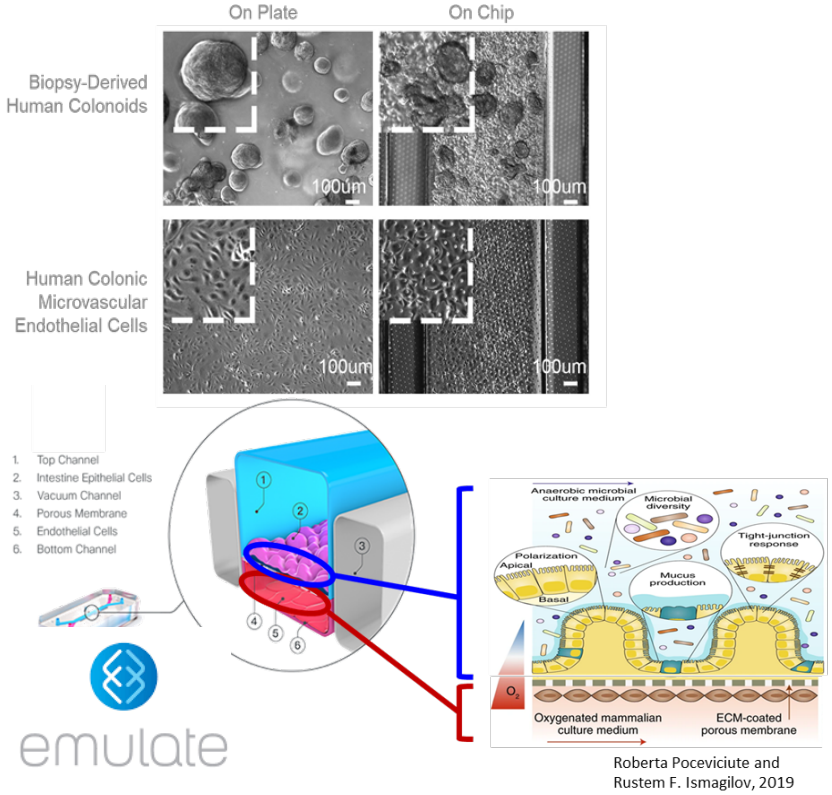
CVM: Evaluating and Qualifying a Human-relevant Model to Assess Effects of Drug Residues on the Human Intestinal Microbiome and Resistome Residents

PI: Daniel A. Tadesse



The effects of drug residues in food-producing animals on the human intestinal microbiome is an important human food safety endpoint of concern that must be addressed during the new animal drug approval process.

Two endpoints of concern

- Disruption of the microbiome colonization barrier, and
- Antimicrobial resistance development among microbiome residents

Goals:

- Investigate Emulate's intestine-on-a-chip system as an alternative *in vitro* model for co-culturing human intestinal cells with gut microbiota
- Use metagenomic and metatranscriptomic approaches to measure changes among microbiome and resistome residents

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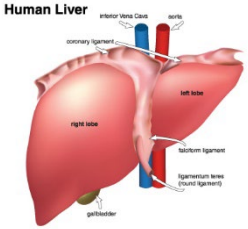
NCTR

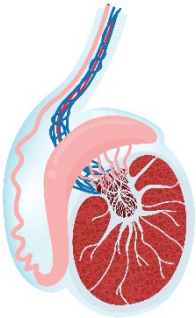
Emulate

- Human liver
- Rat liver
- Brain

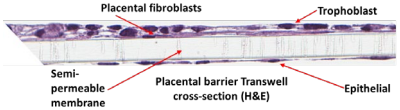


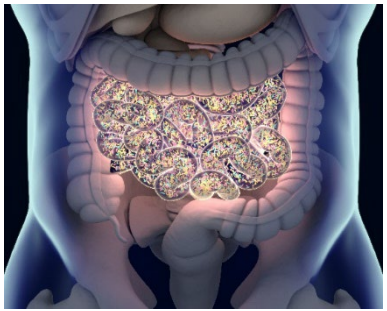
TISSUSE
Emulating Human Biology

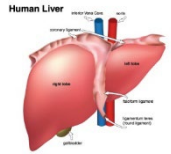
FDA



- Testes
- Placenta
- Human intestine with microbiome







FDA NCTR-CDER-Industry Joint Research in Liver MPS

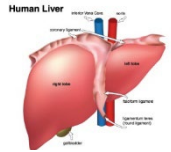
PI: Dr. Qiang Shi

Project Title: Establishment of a liver-chip system to predict individual susceptibility and adaptation to drug-induced liver injury

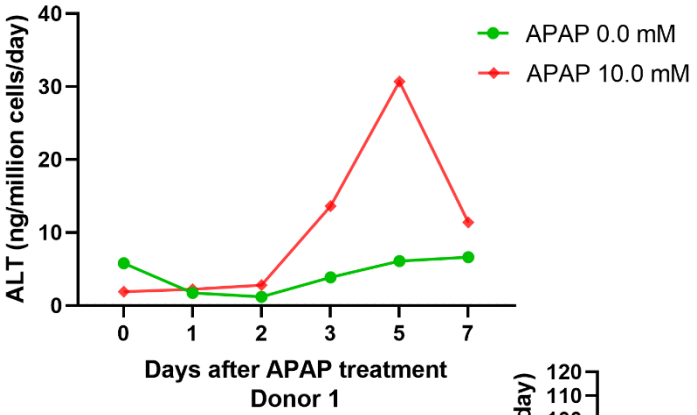
- A Cooperative Research and Development Agreement (CRADA) between the U.S. FDA and Emulate, Inc.

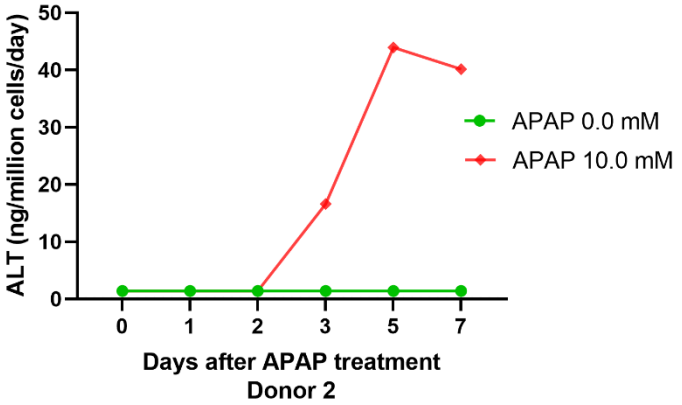
Objectives:

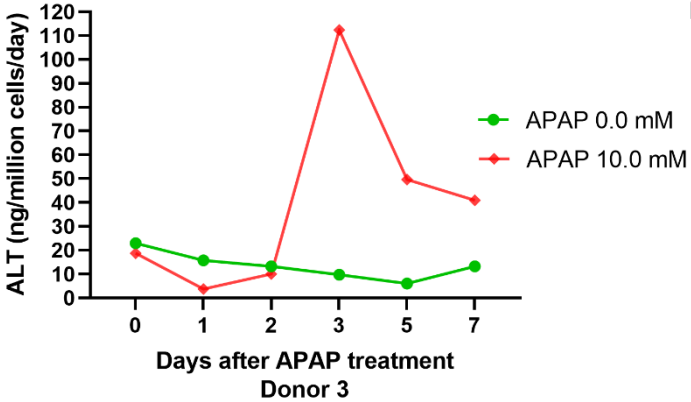
1. To establish a liver-chip model that can characterize transient, adaptive (i.e., benign) and individualized hepatic responses in multiple human cell lines to model the hepatotoxic drug, acetaminophen.
2. To investigate endpoints associated with benign and adverse drug-induced hepatotoxicity.



FDA NCTR-CDER-Industry Joint Research in Liver MPS





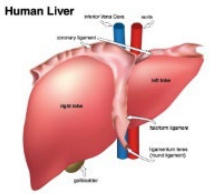


Donor-dependent acetaminophen toxicity in cells cultured in liver-chips

FDA NCTR-CDER-Industry Joint Research in Liver MPS

Ongoing and future efforts:

1. Testing cells from more donors for statistically meaningful results
2. Characterizing the baseline CYP2E1 activity/expression of the cells from the four donors already tested: APAP needs to be metabolized by CYP2E1 to generate the toxic metabolite leading to toxicity, therefore, donor-dependent toxicity may be due to CYP2E1 activity/expressions
3. Discussing collaborations to comprehensively characterize the cells using single-cell RNA-sequencing and subcellular spatial transcriptomics to discover biomarkers that may help predict the donor-dependent responses to acetaminophen



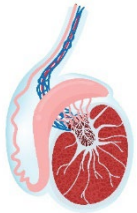
Development of Testicular and Placental Models of Zika Virus (ZIKV) Infection and Transmission

- Rationale: Bloodborne ZIKV is sexually transmissible and capable of crossing the placenta. Infection of developing embryonic neural tissues might result in negative pregnancy outcomes (congenital Zika syndrome, microcephaly, fetal loss)
- Objective: Develop testicular and placental models of ZIKV infection and transmission. Co-culture these models with embryonic neural cells in organ chip co-cultures to evaluate infection
- Approach:
  - Assess ZIKV pathogenic infections and test the efficacy and safety of FDA-regulated IgG products to treat ZIKV infections, complement animal studies
- Impact: Novel *in vitro* models of ZIKV infection might facilitate therapeutic and vaccine development and testing

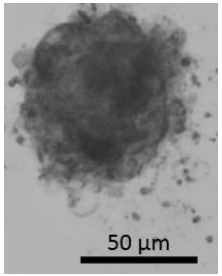
NHP-Based Testicular MPS of ZIKV Sexual Transmission

PIs: Dayton Petibone (NCTR) and Maria Rios (CBER)

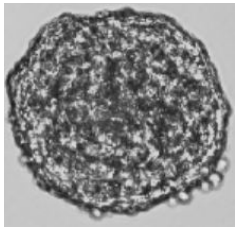
TISSUSE
Emulating Human Biology



NHP-TO

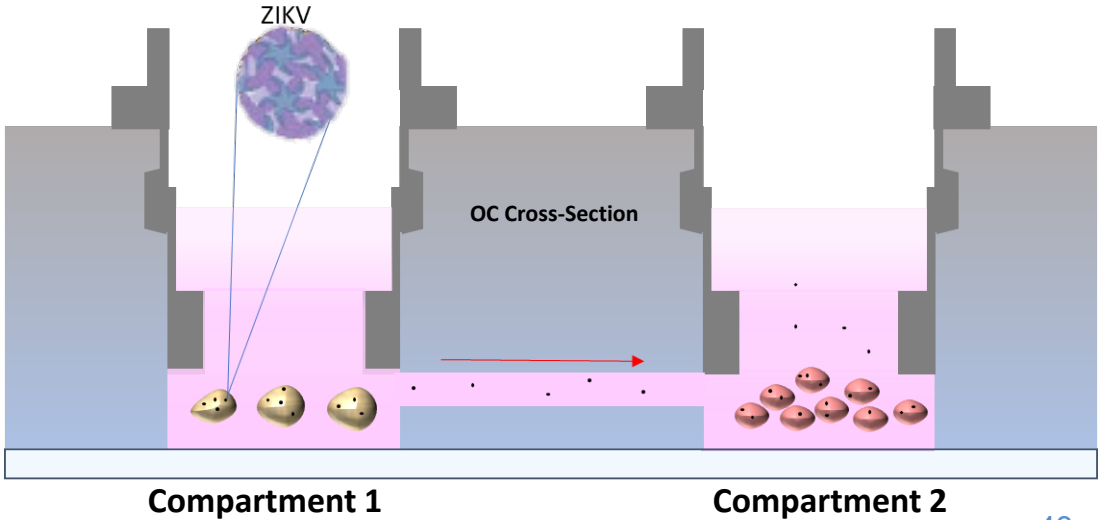


NHP-Neurosphere



NHP-TO & Neurosphere organ chip

- Compartment 1: testicular organoids uninfected or ZIKV infected
- Compartment 2: undifferentiated (stem cells) or differentiated (neurons, astrocytes, oligodendrocytes) neurosphere targets of ZIKV infection
- Monitor ZIKV production, cytotoxicity, metabolic function, cytokine release



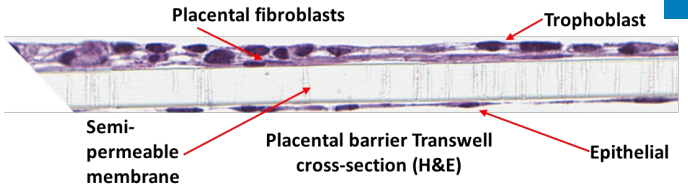
Human-Based Placental Barrier MPS for ZIKV Infection

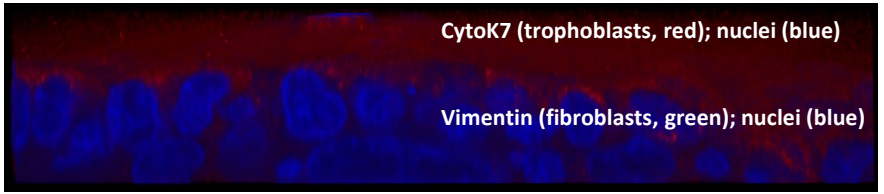
PIs: Evi Struble (CBER) and Dayton Petibone (NCTR)

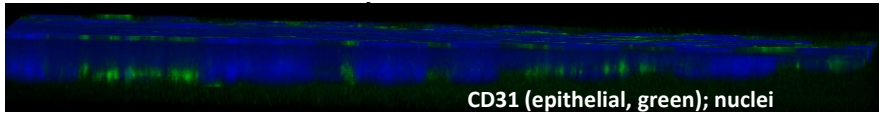
Placental Barrier & Neurosphere Organ Chip

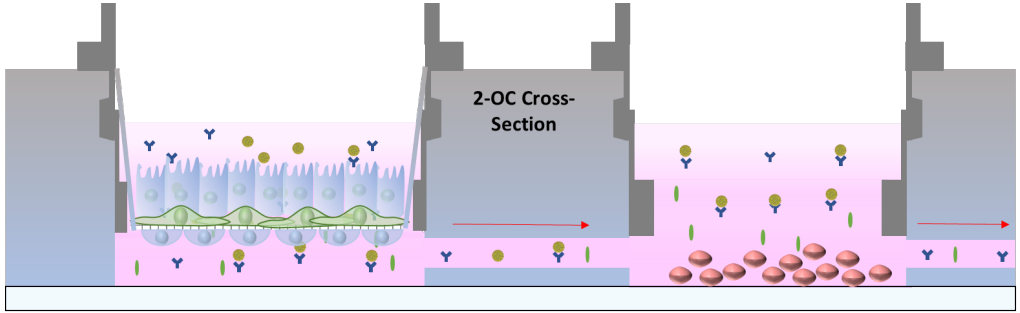
- Compartment 1: Replicate placental barrier
- Compartment 2: undifferentiated (stem cells) or differentiated (neurons, astrocytes, oligodendrocytes) neurosphere targets of ZIKV infection that result in pathology
- Microfluidics replicate villus fetal blood circulation
- Treat placental barrier with ZIKV and IgG and monitor cytotoxicity, metabolic function, transcytosis and pro-inflammatory signaling molecules (PISM)

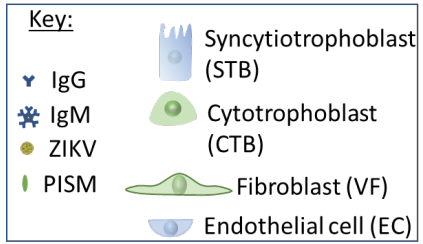
Placental barrier model











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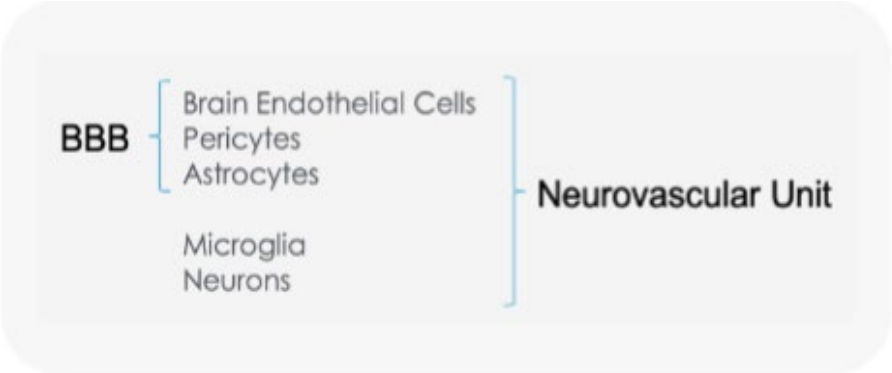
Brain Chip

PI: Dr. Hector Rosas-Hernandez

Chip model:

- Emulate
- Fully isogenic brain chip
  - Human iPSC-derived neurons, astrocytes, microglia and pericytes cultured on brain channel
  - Human iPSC-derived brain microvascular endothelial cells cultured on vascular channel
- Physiological flow and cellular interactions on the brain-chip

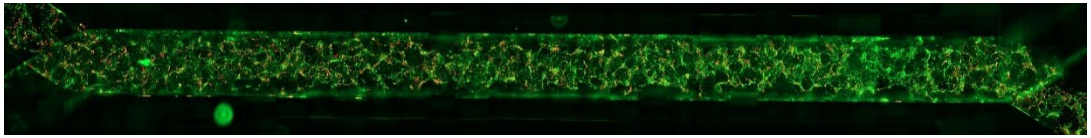


Brain Chip

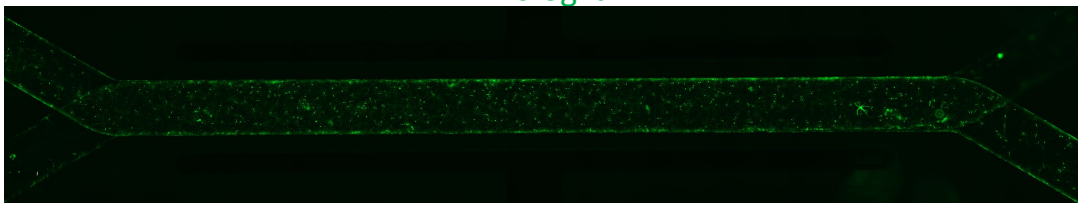
- Disease modeling (neurodegenerative disorders, neuropsychiatric disorders, etc.)
- Study populations of interest (genetic mutations, SNPs, specific genetic backgrounds)
- Study rare diseases (with specific iPSCs from these patients)
- Examine human-specific treatments for which animal models are not an option (monoclonal antibodies for AD, oligonucleotides for ALS, cell therapies, etc.)
- Exposure of drugs/chemicals on a more human-relevant manner (brain cells will only get exposed if the compounds of interest cross the BBB)

Human iPSC-derived Brain Cells Cultured on the Brain-chip

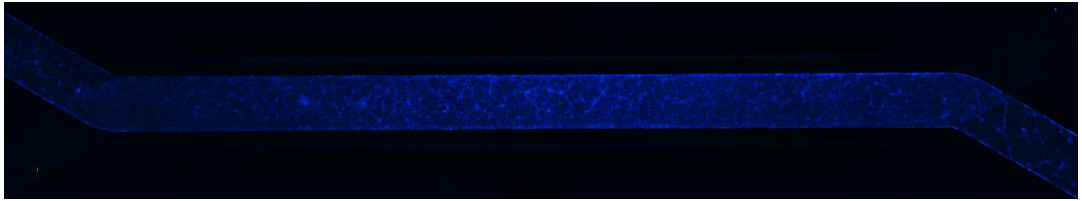
iNeurons and iAstrocytes



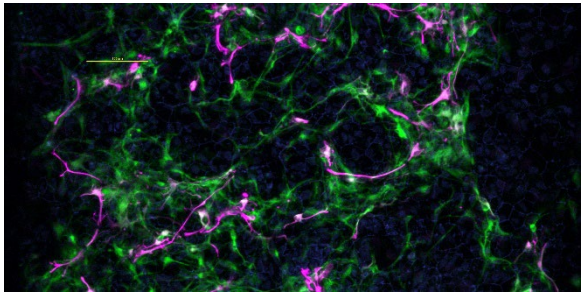
iMicroglia



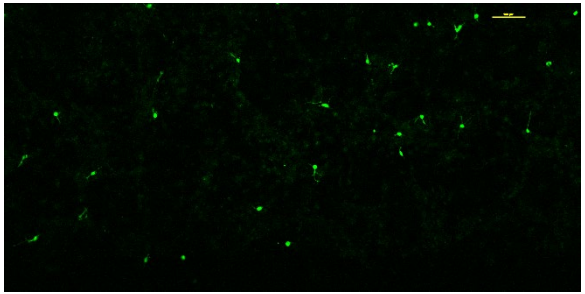
iBrain Microvascular Endothelial Cells



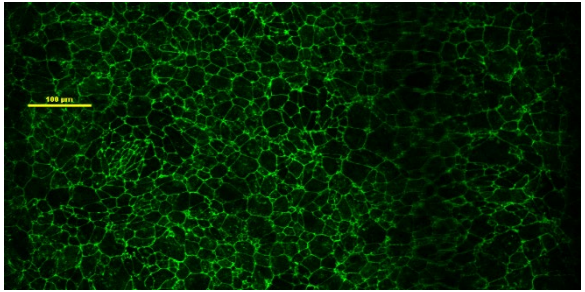
iNeurons and iAstrocytes



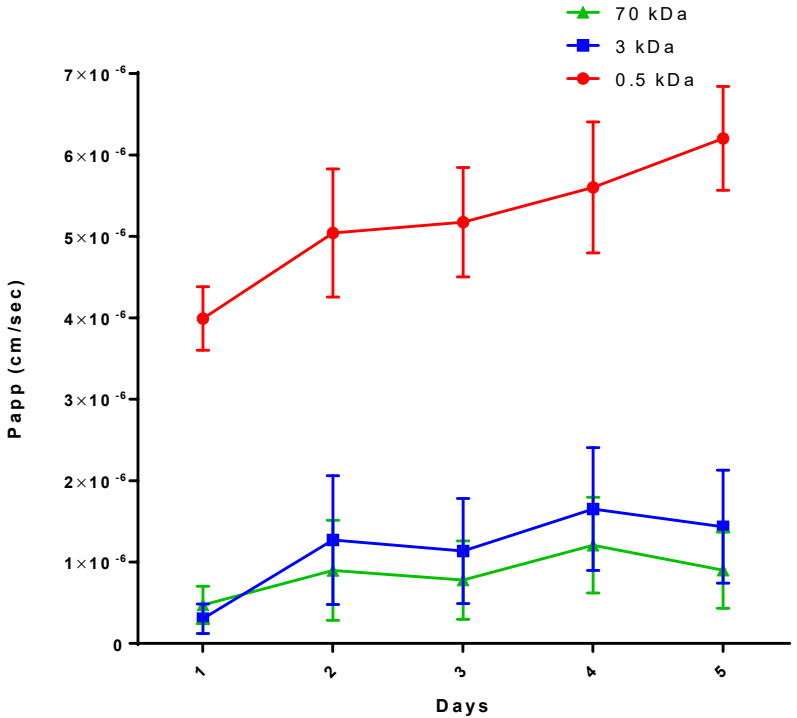
iMicroglia



iBrain Microvascular Endothelial Cells



Endpoints



Source of Variation	% of total variation	P value	P value summary
Interaction	0.7075	0.9735	ns
Time	2.805	0.0751	ns
Size	50.44	< 0.0001	****

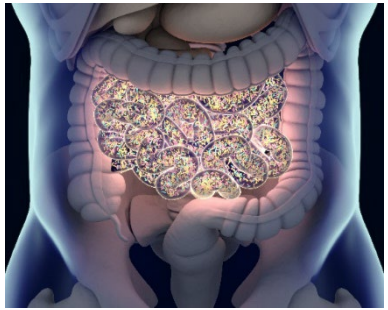
- **Paracellular permeability**
  - Stable at least for 5 days in culture
  - Capable to differentiate size-dependent permeability of molecules to the “brain”
- **P-gp function**
  - Efflux of molecules/chemicals/drugs from the brain back to the “bloodstream”
- **Cell death/apoptosis**
- **Neuroinflammatory markers**
  - ELISA, multiplex assays
- **Expression of proteins of interest in both brain and vascular channels**
  - Western blot, ELISA, mRNA
- **Accumulation and clearance of proteins of interest**
  - Relevant for modeling neurodegenerative disorders

→ Stable permeability for at least 5 days
→ Capable to differentiate size-dependent permeability

Human Intestine and Microbiome

- Drs. Sangeeta Khare and Kuppan Gokulan

- Will examine the impact of xenobiotics on human intestinal tissues and organoids looking at both the host and microbiome level



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  - Kuppan Gokulan


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