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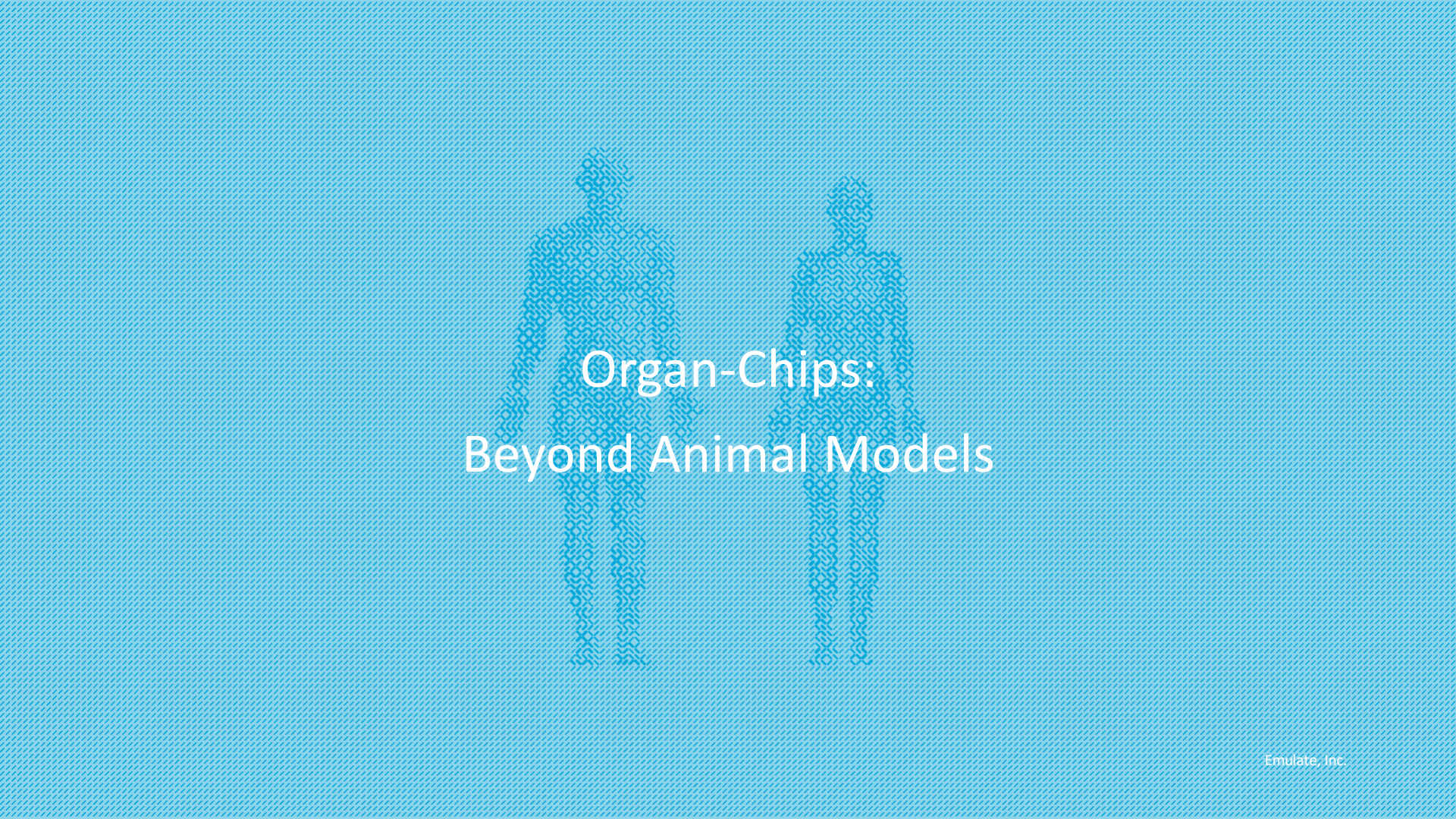
Beyond Animal Testing:
Development of Organs-on-Chips to Emulate Human Biology

Lorna Ewart, PhD
Executive VP European Scientific Liaison

Our Past and Present


Organ-Chips:
Beyond Animal Models
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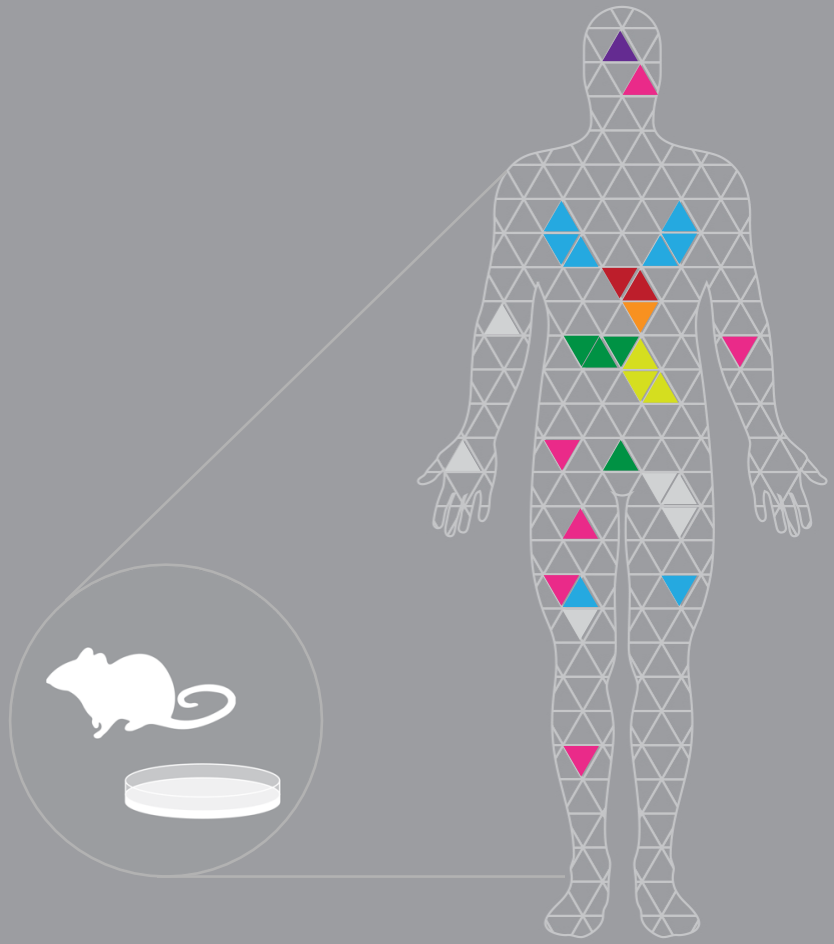

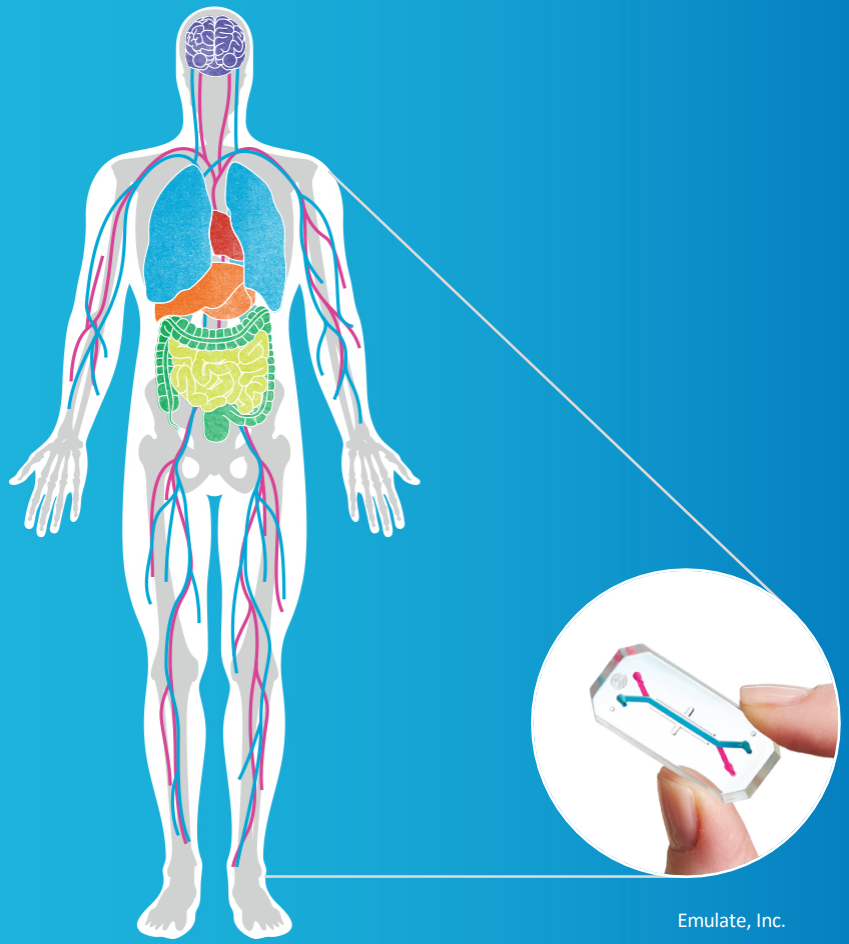

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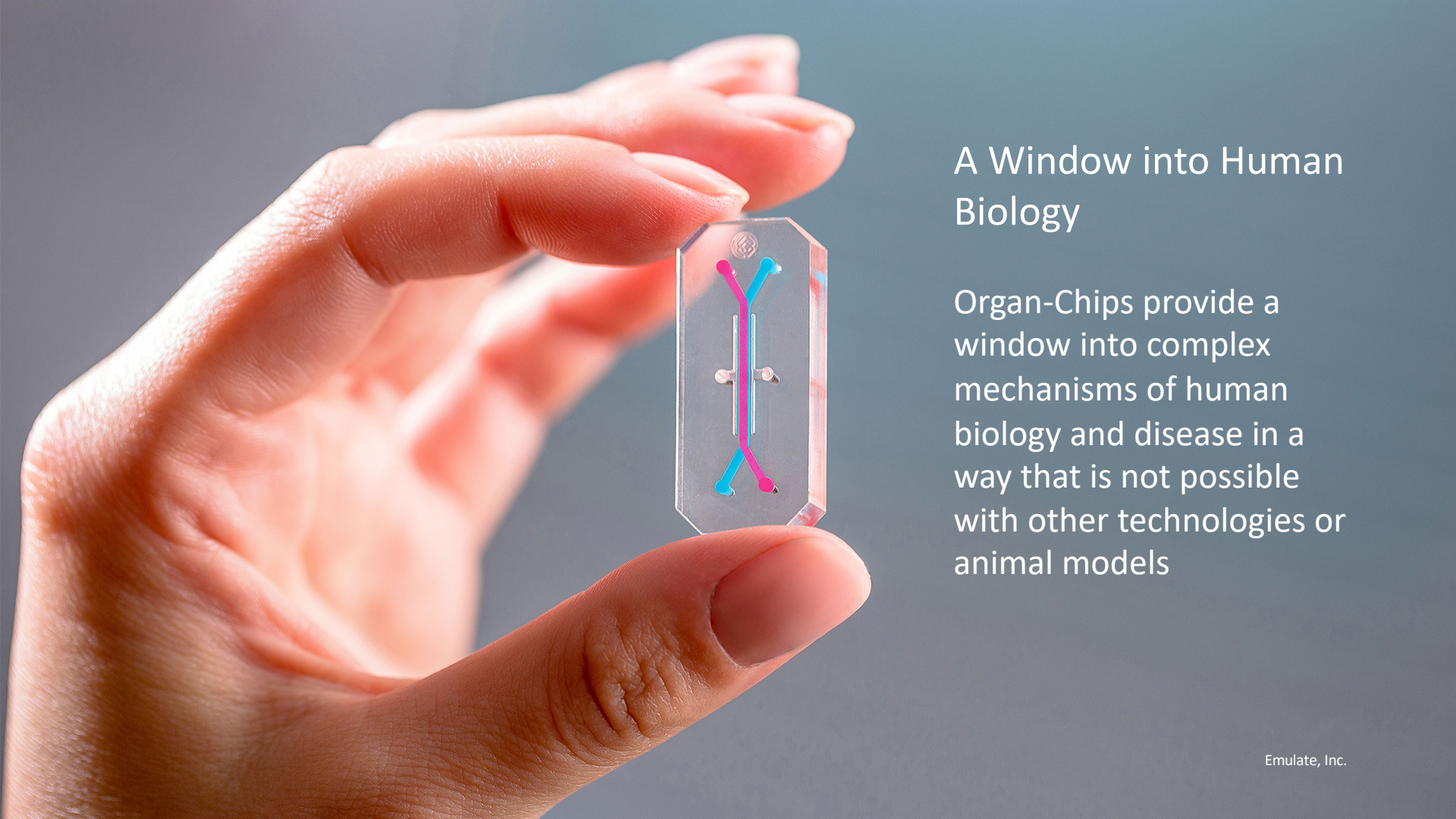

A Window into Human Biology

Organ-Chips provide a window into complex mechanisms of human biology and disease in a way that is not possible with other technologies or animal models

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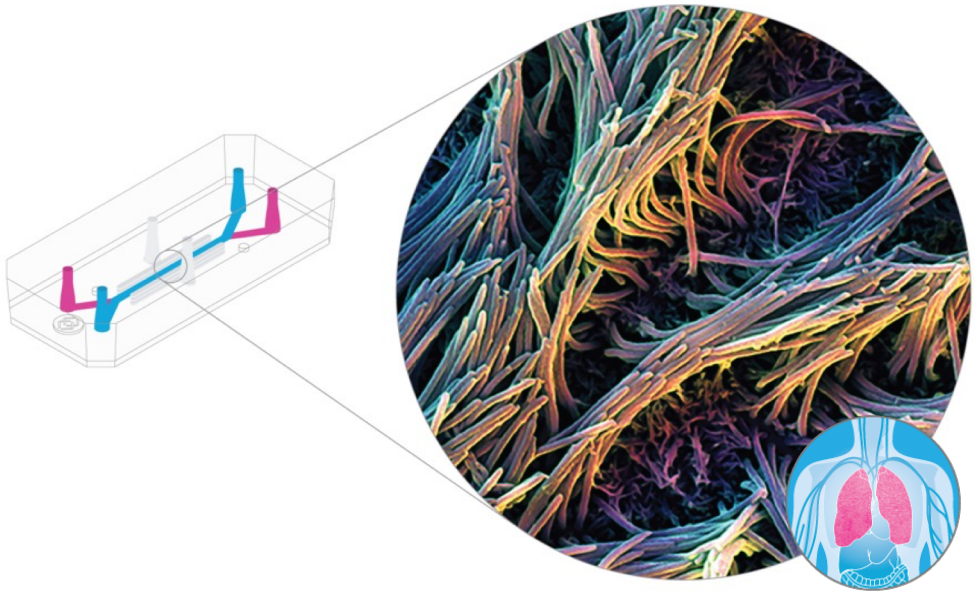
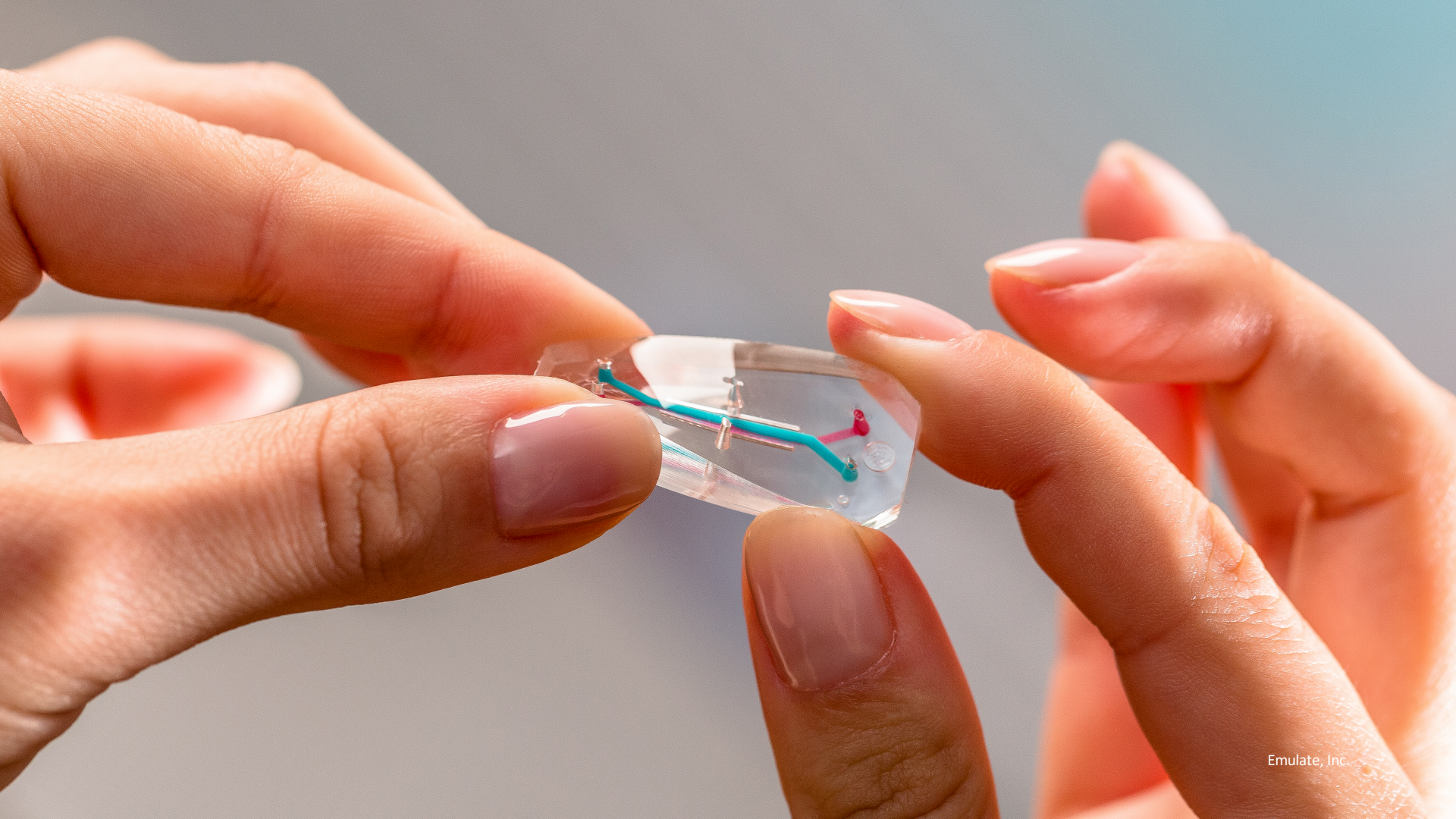
Recreating the Cellular Microenvironment in Our Chips

- Extracellular matrix and cell interactions
- Cell shape and cytoarchitecture
- Tissue-tissue interactions
- Mechanical forces
- Dynamic system — flow
- Resident or circulating immune cells

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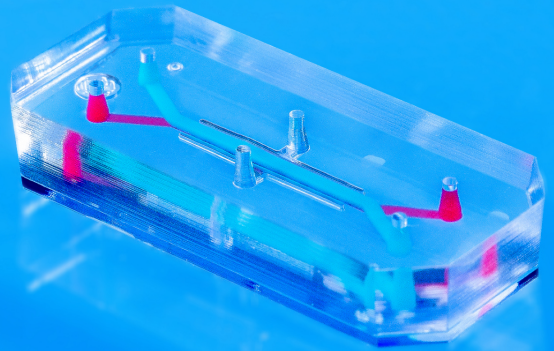
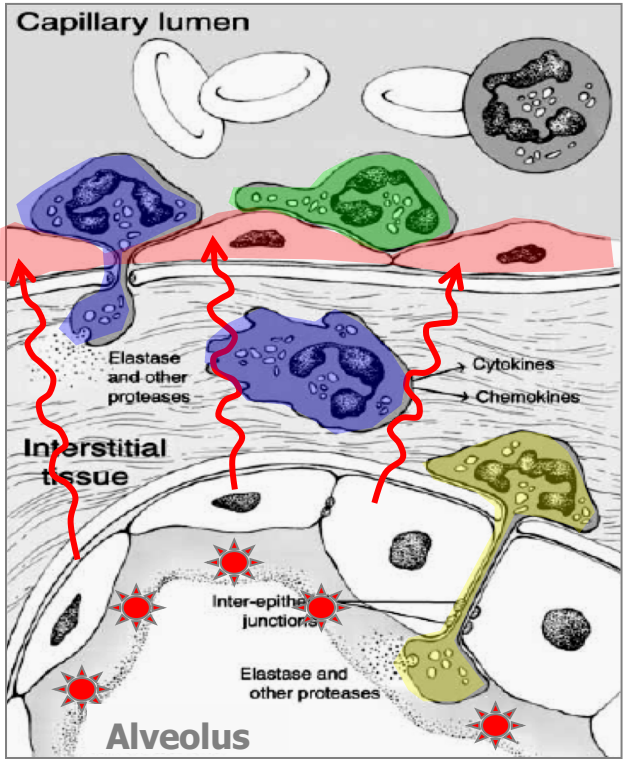
Lung-Chip video link: https://vimeo.com/267637620

LUNG-CHIP



Lung inflammation



Endothelial activation (express surface ICAM-1) → Leukocyte adhesion

Leukocyte adhesion → Diapedesis & transmigration

Diapedesis & transmigration → Infiltration into alveoli

Infiltration into alveoli → Pathogens

Pathogens → Pro-inflammatory cytokines

Pro-inflammatory cytokines → Endothelial activation (express surface ICAM-1)

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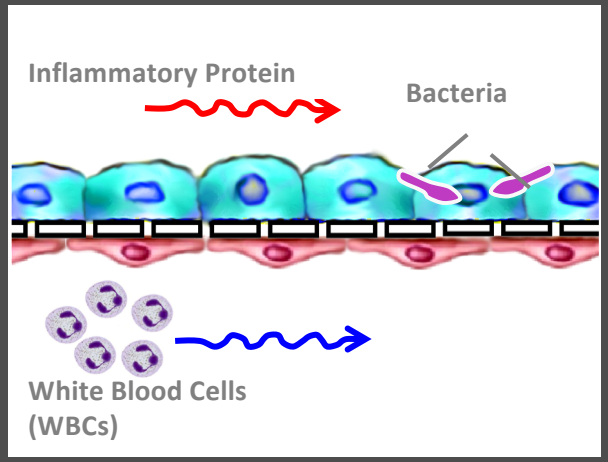
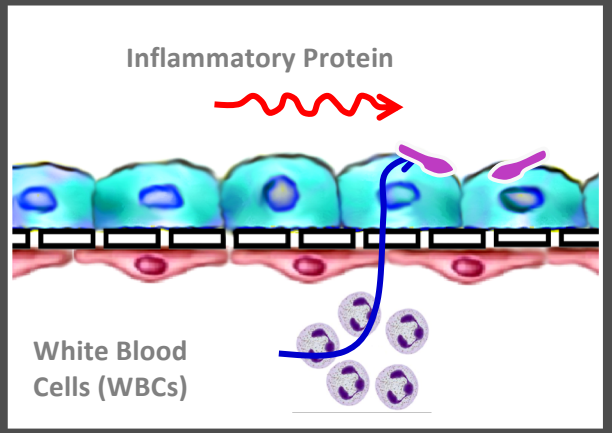
Lee & Downey, *Am J Respir Crit Care Med* 2001

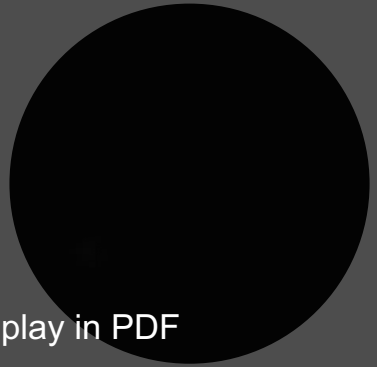
Inflammation and Immune System Interaction in Lung-Chip: Recapitulating Biological Complexity





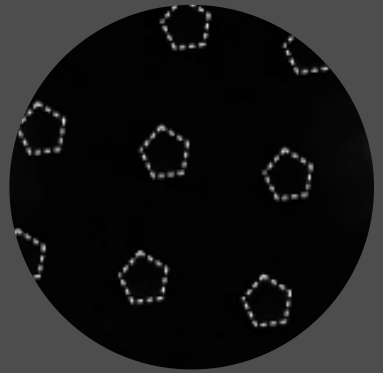
Control

Inflamed

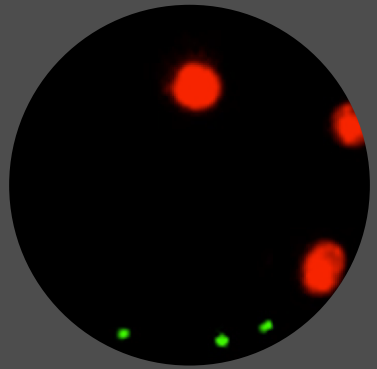


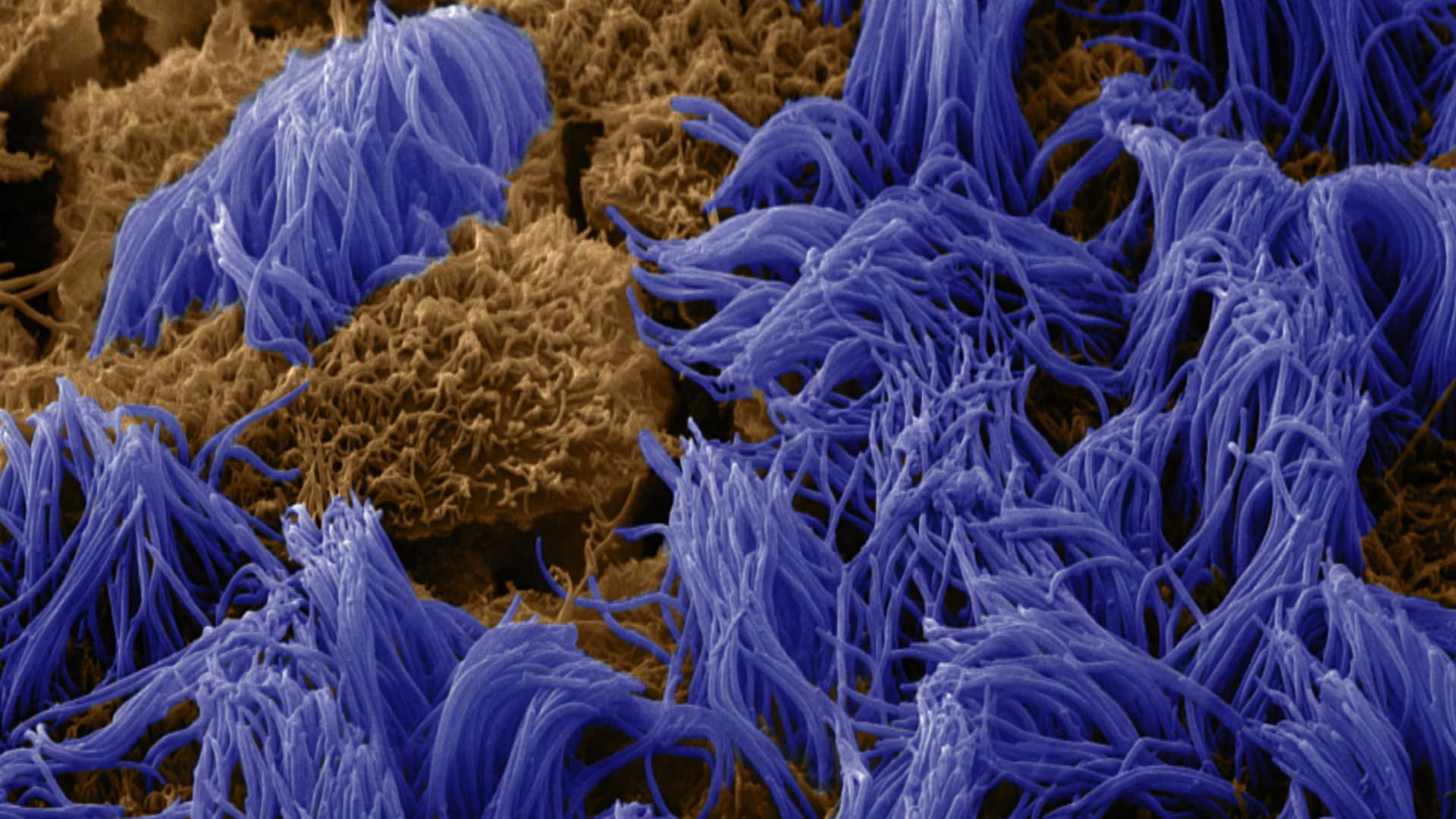
Videos won't play in PDF

WBC Transmigrating

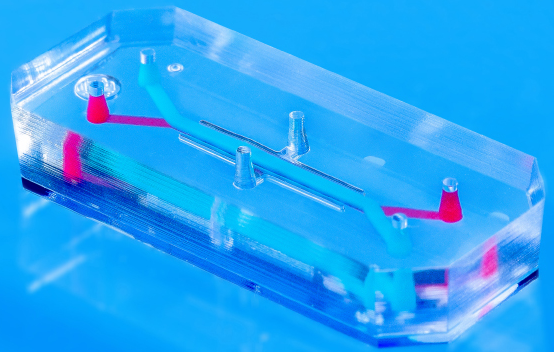


WBC Killing Pathogens





LIVER-CHIP




Science
Translational
Medicine
6 NOVEMBER 2019
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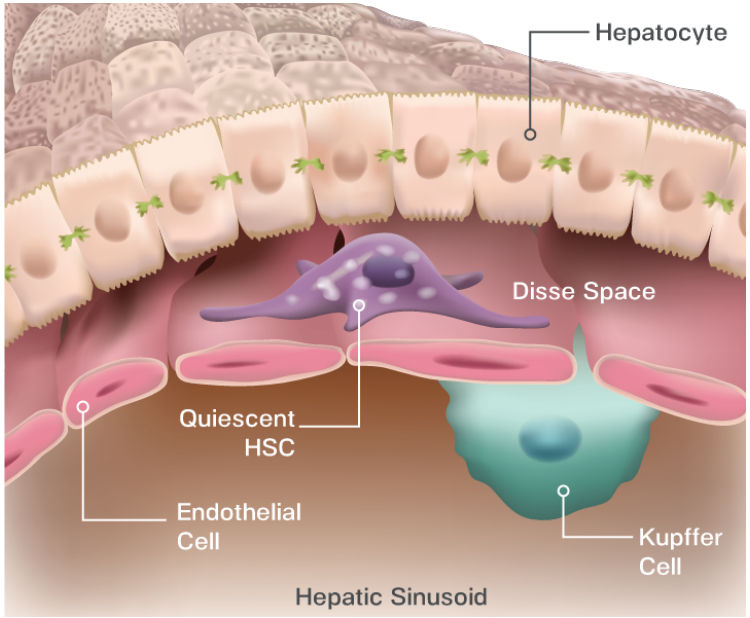
Liver-Chip: Building Tissue Complexity



J Clin Invest. 2007; 117(3):539-548.

Co-culture

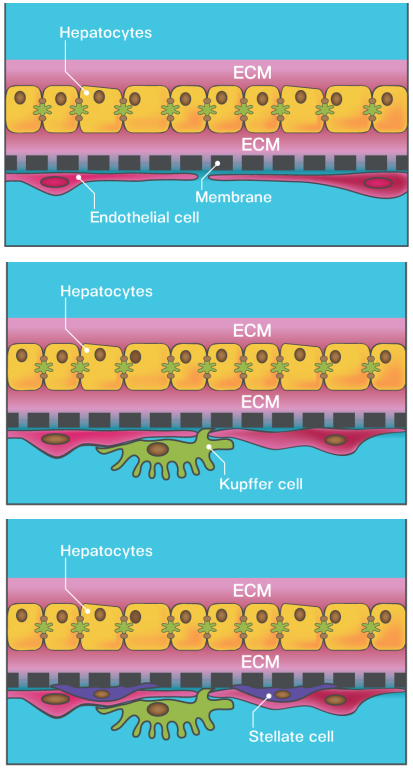
Hepatocytes + Liver Sinusoidal Endothelial Cells (LSECs)

Tri-culture

Hepatocytes + LSECs + Kupffer cells

Quad-culture

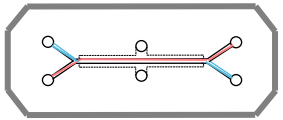
Hepatocytes + LSECs + Kupffer cells + Stellate cells

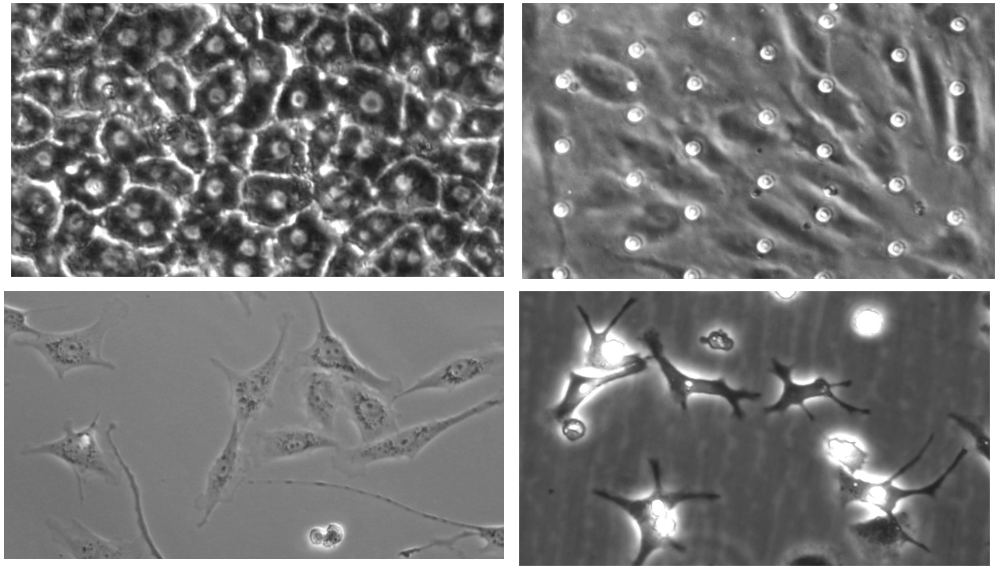


Liver-Chip Developed and Validated with Multiple Partners

- **Co-culture (Hepatocyte + Liver Sinusoidal Endothelial cells)**
- **Tri-culture (Hepatocyte + Liver Sinusoidal Endothelial cells, Kupffer)**
- **Quadruple-culture (Hepatocyte + Liver Sinusoidal Endothelial cells, Kupffer, Stellate cells)**

- Human Liver-Chip
- Rat Liver-Chip
- Dog Liver-Chip





FDA

Johnson & Johnson

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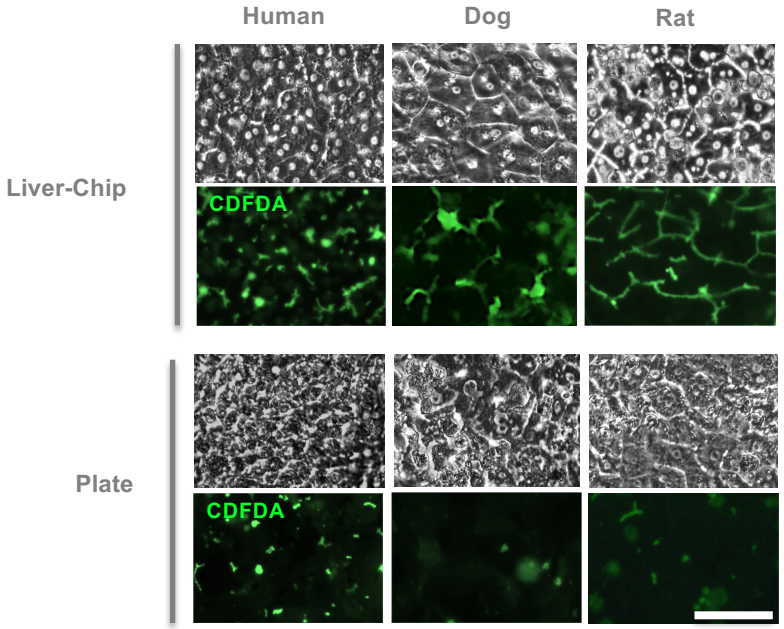
Liver-Chip: Morphology

Day 14 in culture



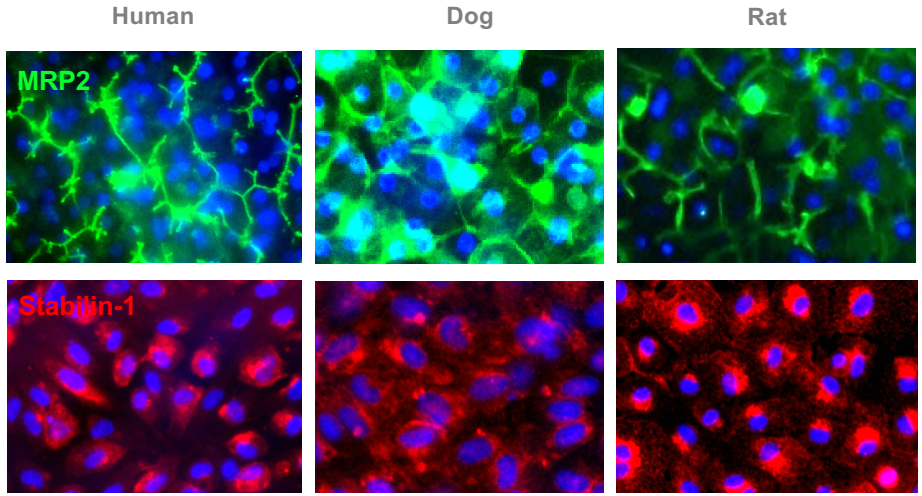
5(6)-Carboxy-2′,7′-dichlorofluorescein diacetate (CDFDA)

bar, 100 µm

Hepatocytes in three species Liver-Chips maintained their stereotypical *in vivo*-like morphologies and formed improved bile canalicular networks compared to plate sandwich culture over 2 weeks in culture

Liver-Chip: Morphology

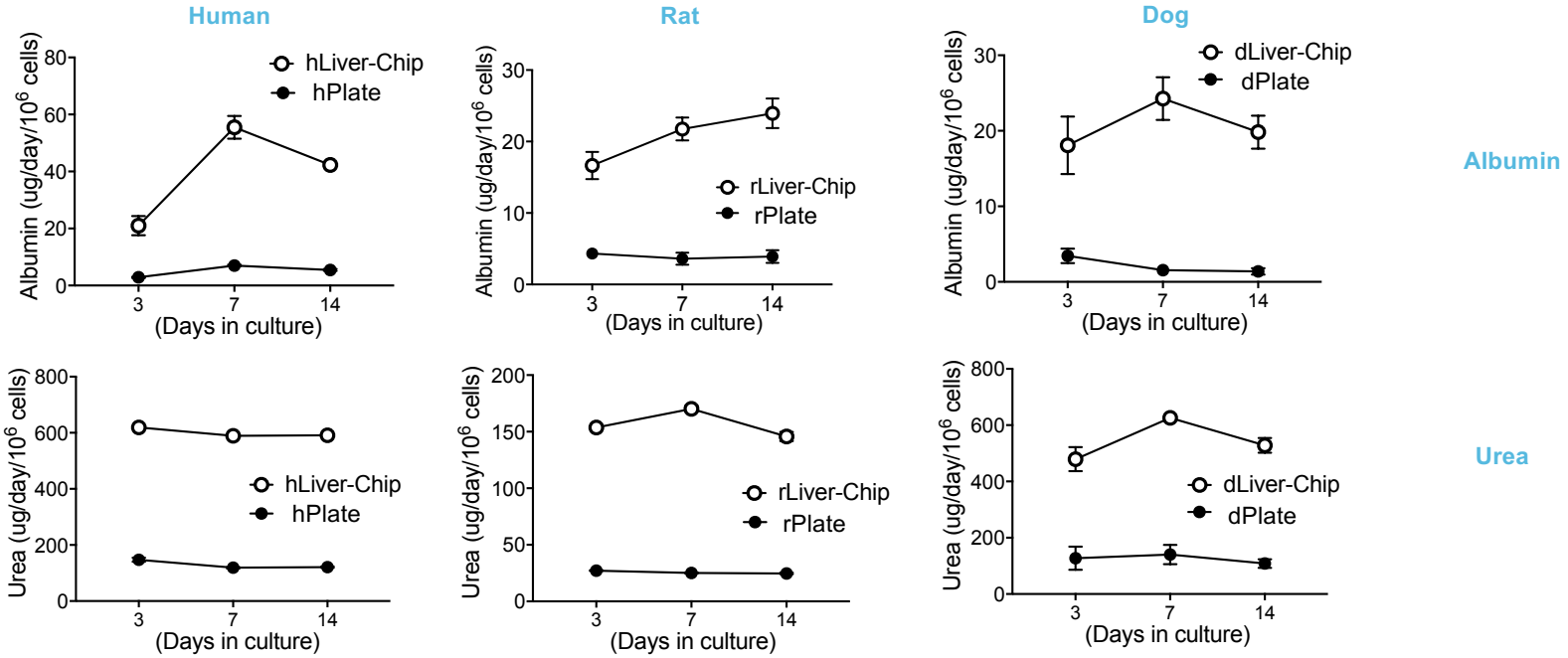
Day 14 in culture



MRP2:
multidrug resistance-associated protein 2

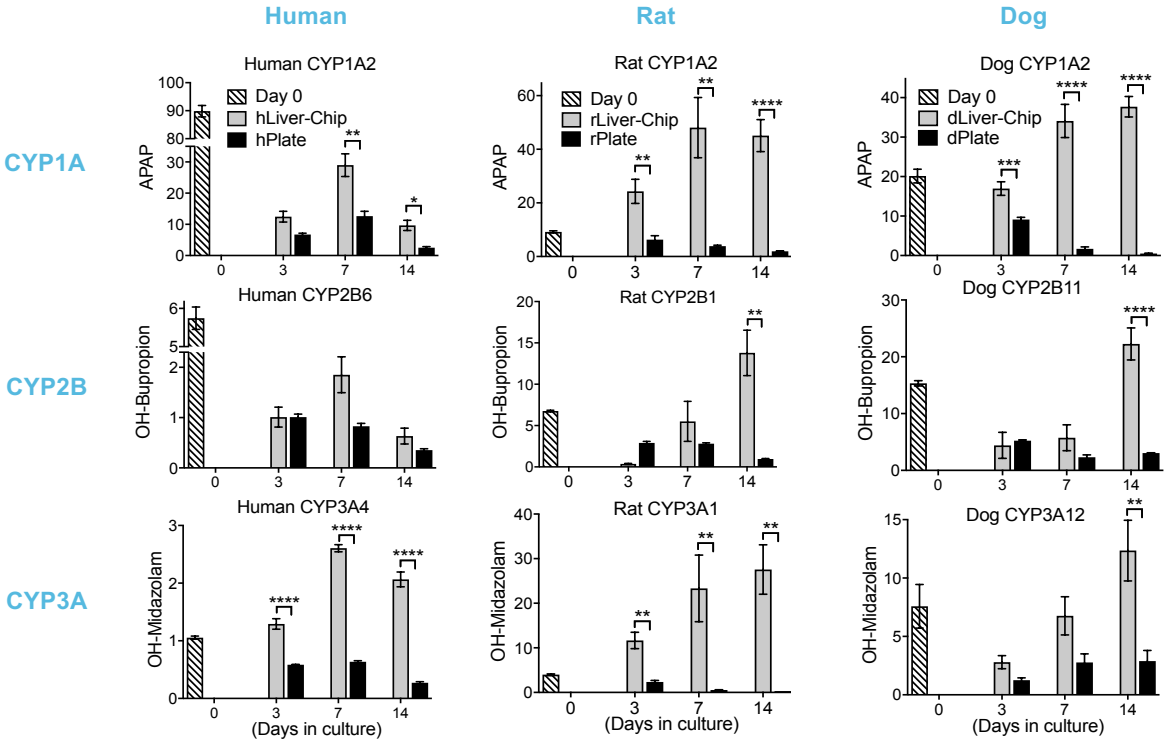
Characteristic branched bile canalicular networks in hepatocytes over 2 weeks in culture in the Liver-Chips.
Expression of the multifunctional scavenger receptor stabilin-1, which is expressed selectively on sinusoidal endothelial cells and is critical for hepatic clearance in LSECs in the Liver-Chips.

Liver-Chip: Albumin and Urea Secretions



Human, Rat and Dog Liver-Chips maintain high level of albumin and urea secretions over 2 weeks in culture

Liver-Chip: CYP450 Enzyme Activities



Unit : pmol/min/10^6 cells
* p<0.1, ** p<0.01, *** p<0.001, **** p<0.0001

Demonstrated CYP1A, 2B, and 3A activity maintained over 2 week culture

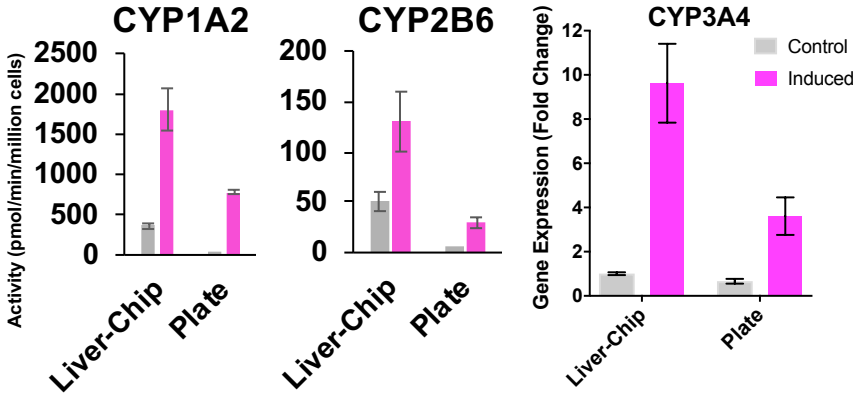
Liver-Chip: CYP450 Induction for Drug-Drug Interactions

Use of prototypical inducers to evaluate the induction potential by measuring CYP450 enzyme activity and gene expression

Selected compounds
- CYP1A inducer: Omeprazole, 50µM
- CYP3A inducer: Rifampicin, 10µM
- CYP2B inducer: Phenobarbital, 1mM

Model
- Co-culture Liver-Chip
- Plate

Read-out
- CYP450 enzyme activity
- Gene expression

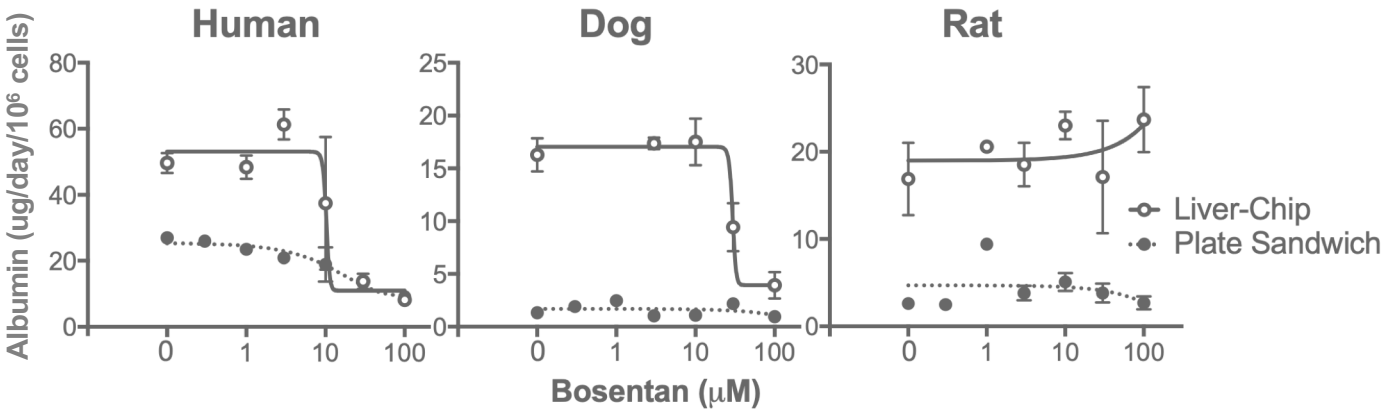


CYP1A2 induction using omeprazole, activity through acetaminophen
CYP2B6 induction using phenobarbital, activity through 4-hydroxycyclophosphamide

Successfully showed ability to induce CYP1A2, CYP2B6, and CYP3A4 enzymes after treatment with prototypical CYP450 inducers in human Liver-Chips

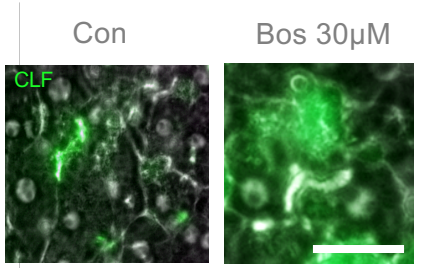
Predicting Species Differences and MOA in Liver Toxicity

Effect of Bosentan on Albumin Secretion

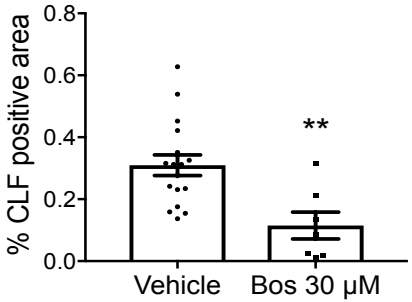


Demonstrated species differences in the hepatotoxicity at *in vivo* relevant doses of Bosentan.
Albumin data shows dose response effects for dog and human, but not for rat, which correlates with *in vivo data:* human > dog > rat

Inhibition of BSEP Transporter



Cholyl-lysyl-fluorescein (CLF)
BSEP substrate



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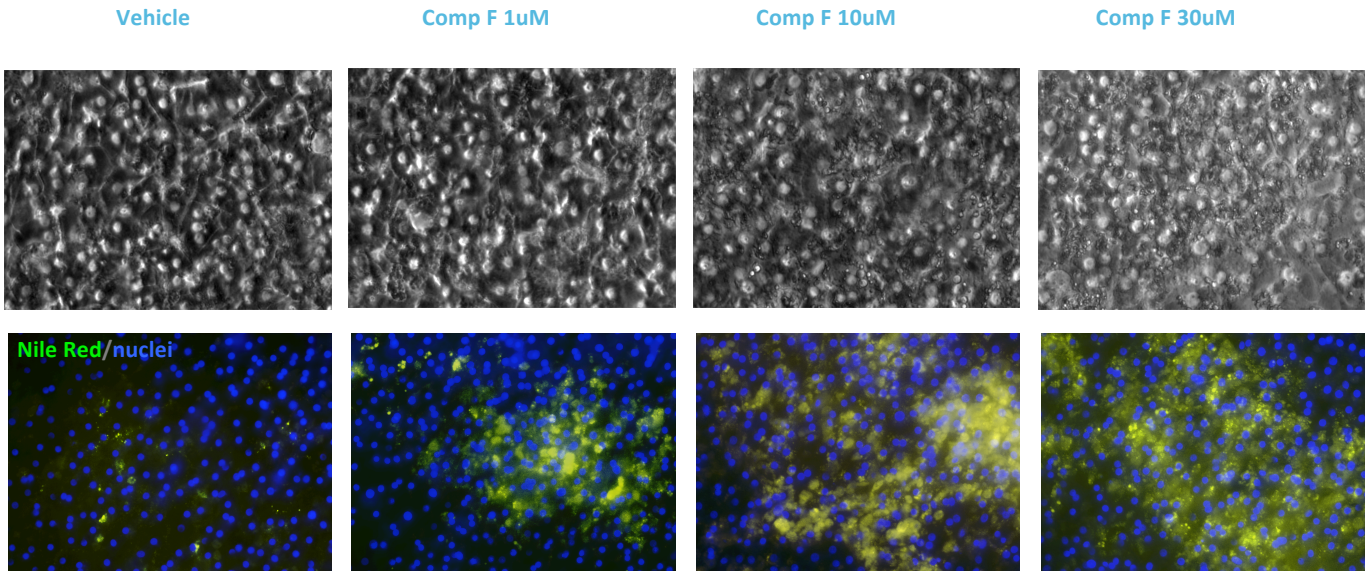
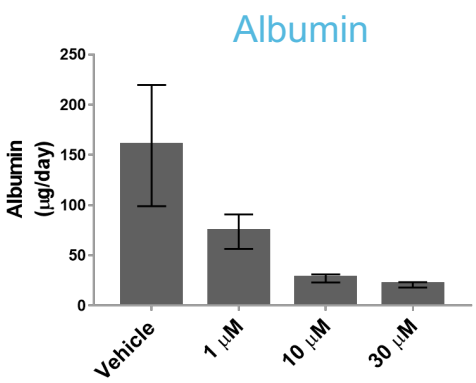
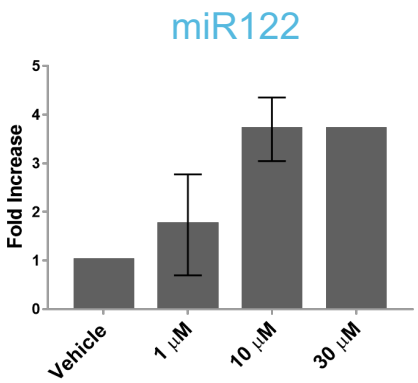
Predicting Human Safety Risks with Liver-Chip

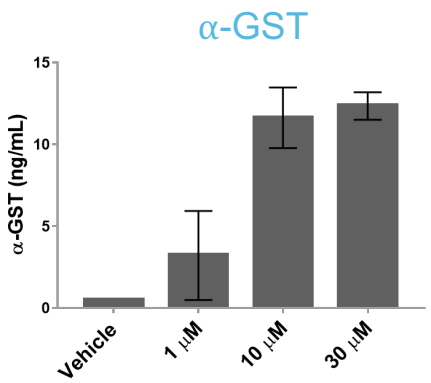
Compound F caused unexpected toxicity in clinical trial leading to death patients from liver failure.
Toxicity not predicted in preclinical animal safety studies

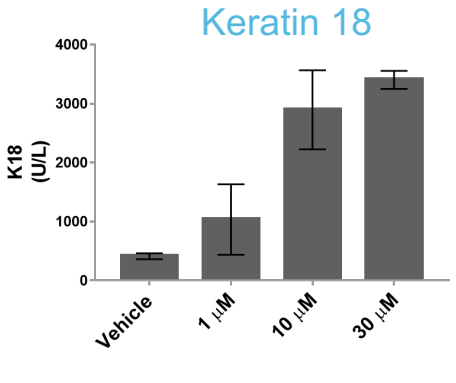


Predicting Human Safety Risks with Liver-Chip





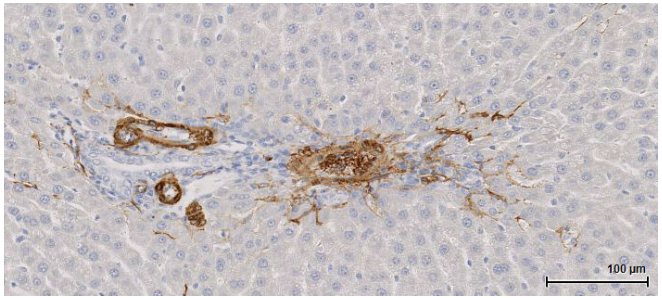




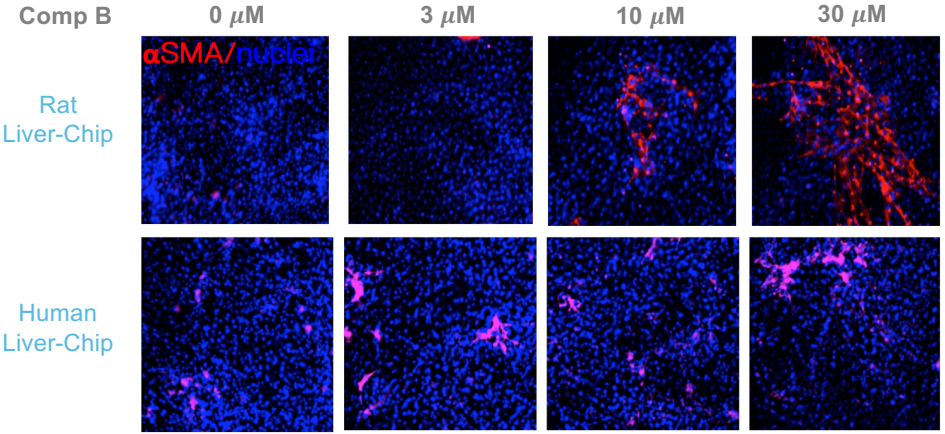
Liver-Chip predicted human liver toxicity of compound F observed in clinical trial and provided additional mechanistic data

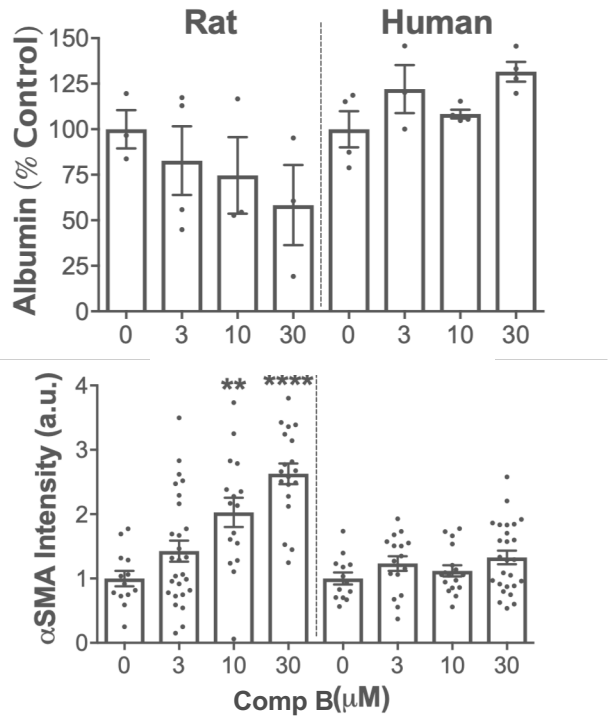
Liver-Chip: Stellate Cell Activation and Species Difference in Toxicity

Rat liver
αSMA



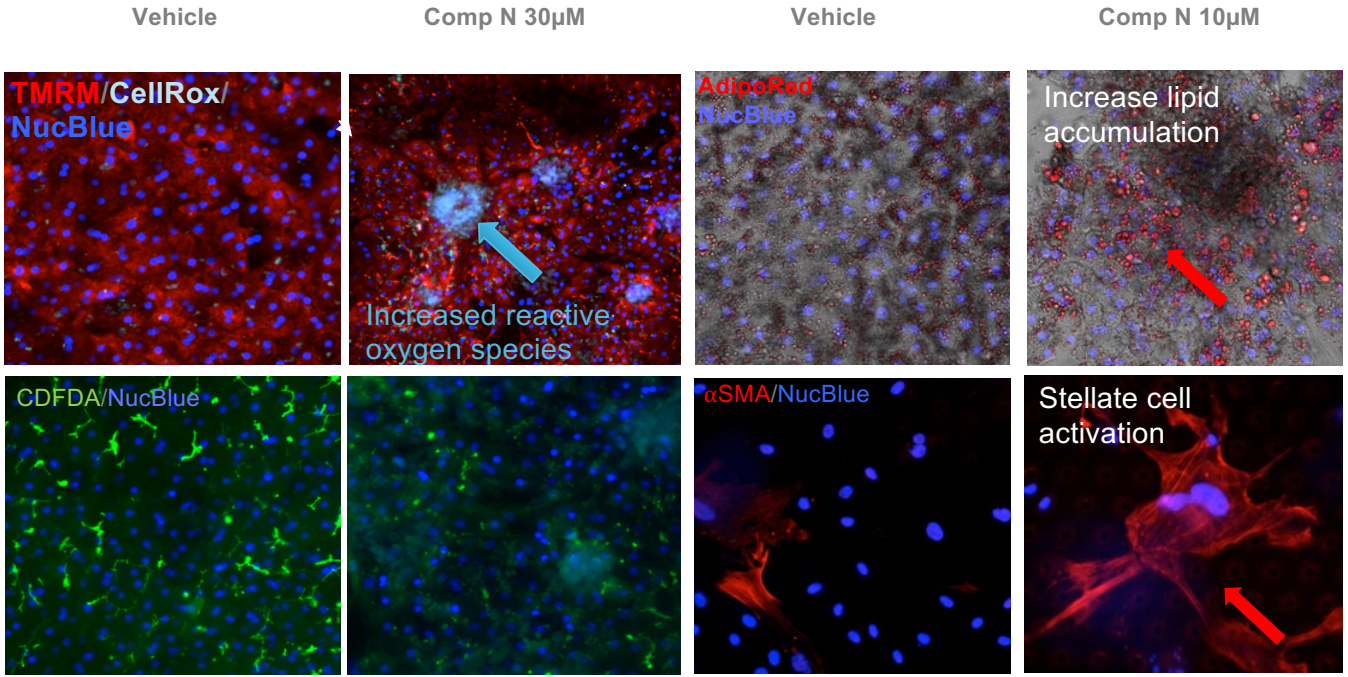
Comp B: discontinued due to fibrosis in rats





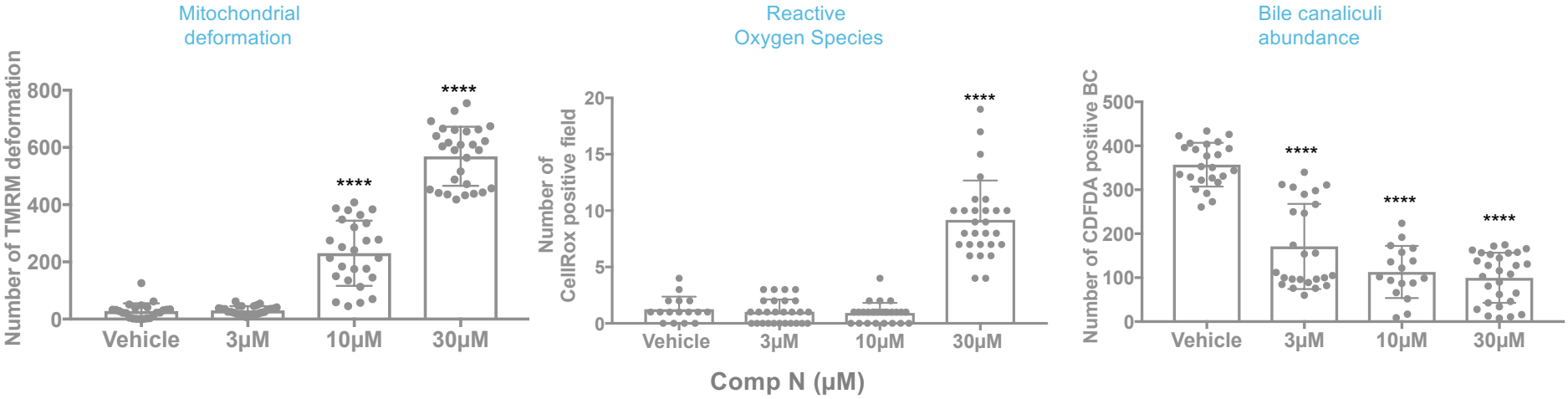
Rat Liver-Chip data correlated with rat *in vivo* data

Liver-Chip Predicted Toxicity Observed in Phase III Clinical Trial



Able to measure multiple functional outputs.
Richness of the data enabled understanding of the complex biology driving the toxicity

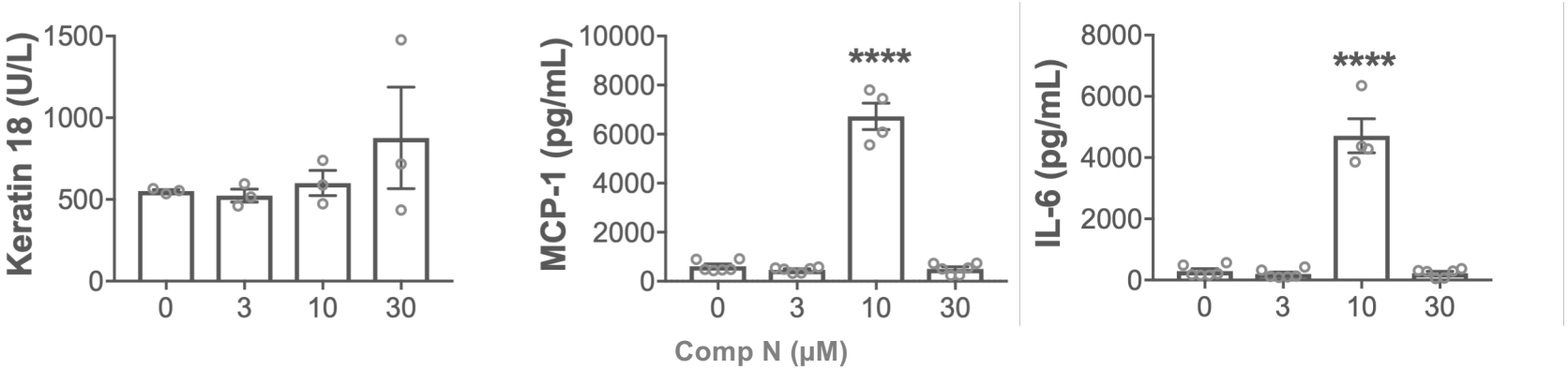
Liver-Chip Predicted Toxicity Observed in Phase III Clinical Trial



Key mechanisms of action driving liver toxicity *in vivo* recapitulated in the Liver-Chip

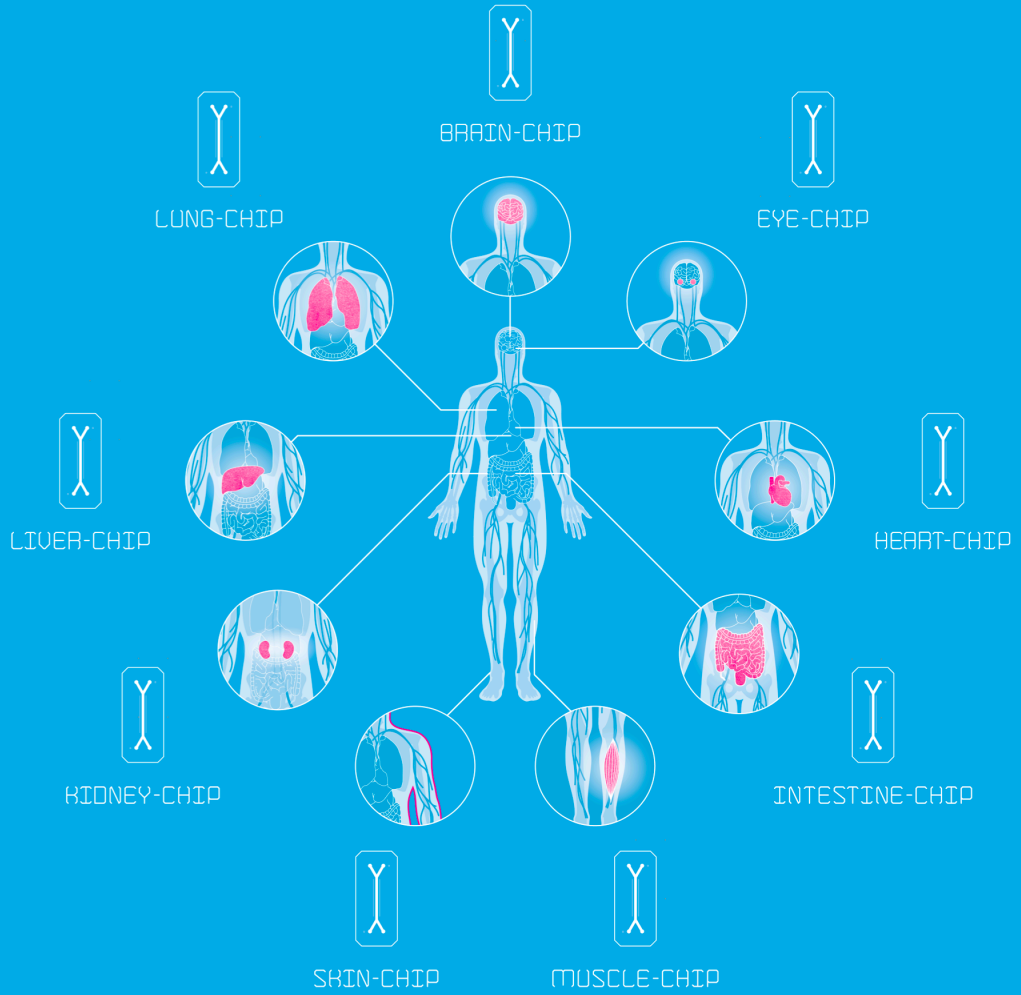
Liver-Chip predicted toxicity of drug that failed at Phase III clinical trials not predicted in preclinical animal safety studies

Liver-Chip Predicted Toxicity Observed in Phase III Clinical Trial



Treatment with Comp N caused significant release of the inflammatory cytokines MCP-1 and IL-6 at 10 μM but not at 30 μM; due to overt cell loss / death at higher concentration

Democratization of the Technology


BRAIN-CHIP
LUNG-CHIP
EYE-CHIP
LIVER-CHIP
HEART-CHIP
KIDNEY-CHIP
INTESTINE-CHIP
SKIN-CHIP
MUSCLE-CHIP

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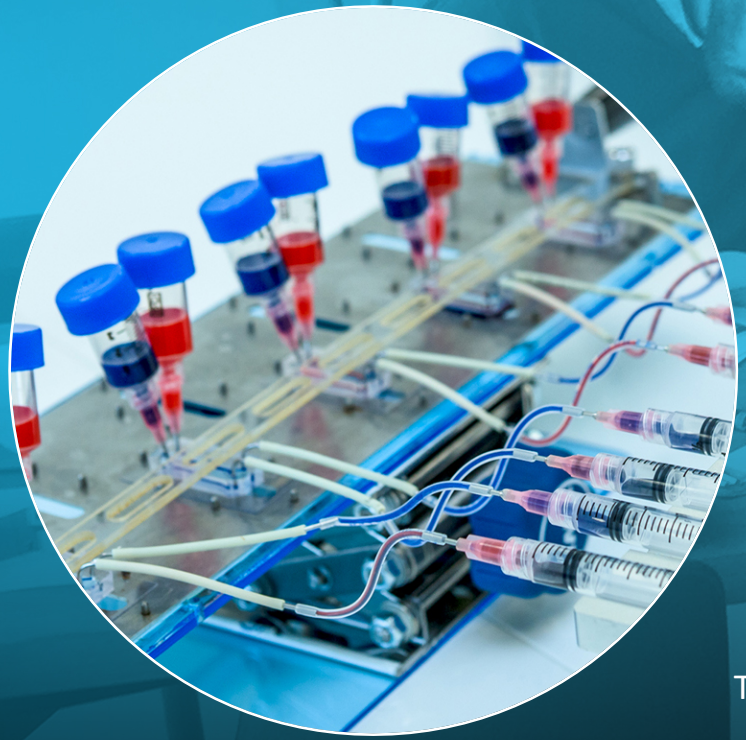

Traditional experimental setup for Organ-Chips

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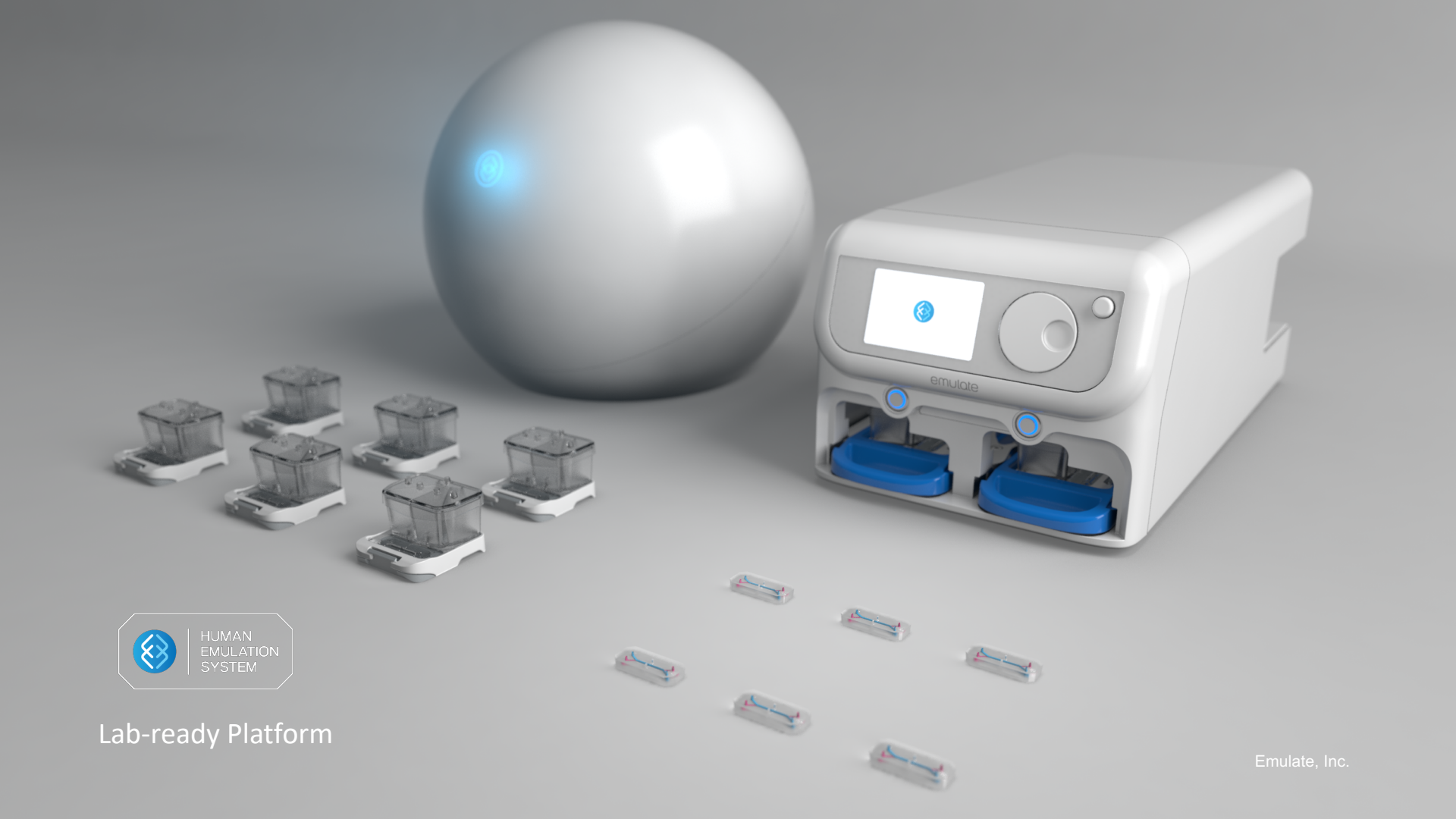


HUMAN
EMULATION
SYSTEM

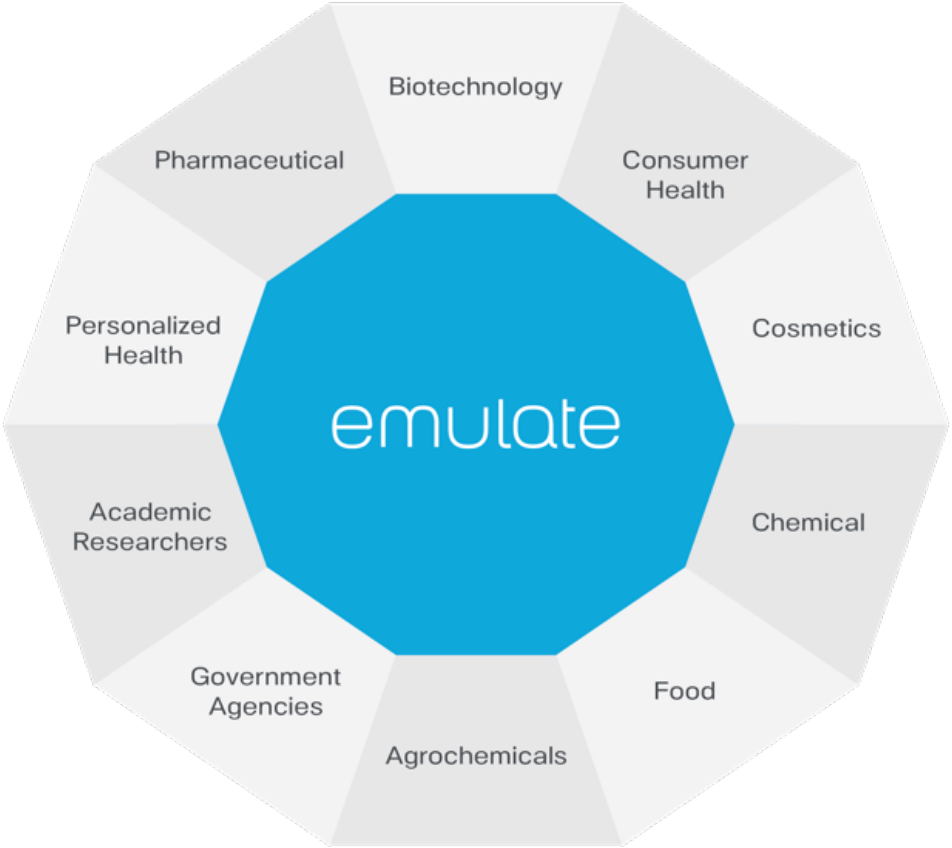
Lab-ready Platform

Emulate, Inc.

Is it effective & safe

How does it work

Is it worth developing



Driving Adoption and Impact on 3Rs: Data, Collaboration, and Partnerships Industry, Government, Academia

FDA Collaboration: CRADA

FDA Commissioner talks about Emulate's Organ-on-Chips platform in his address to the 2017 Science Forum.

"FDA recently signed a research and development agreement with Emulate to explore groundbreaking approaches for toxicity evaluation – using human organ systems in miniature, micro-engineered chips – to study the potentially harmful chemical and biological hazards in foods, dietary supplements, and cosmetics."

Dr. Gottlieb's Remarks to the 2017 FDA Science Forum
May 31, 2017

Link to a transcript of FDA Commissioner's Remarks
https://www.fda.gov/NewsEvents/Speeches/ucm561256.htm

Official Press Release

April 11, 2017

FDA Signs Collaborative Agreement with Emulate, Inc. to Use Organs-on-Chips Technology as a Toxicology Testing Platform for Understanding How Products Affect Human Health and Safety

Cooperative Research and Development Agreement (CRADA) to advance and qualify 'Human Emulation System' to meet regulatory evaluation criteria for product testing

Link to Official Press Release
https://emulatebio.com/press/fda-collab-agreement-emulate/

NBC NEWS

Tiny 'Organ Chips' Promise Big Boost to Testing of Food, Drugs
Tiny replicas of organs could help doctors and scientists learn about how certain foods, chemicals, and dietary supplements affect the human body, the Food and Drug Administration (FDA) says.

Blog from FDA

'Organs-on-Chips' Technology: FDA Testing Groundbreaking Science

Link to FDA Blog
https://blogs.fda.gov/fdavoice/index.php/2017/04/organs-on-chips-technology-fda-testing-groundbreaking-science/

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