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Progress and next steps in making organ-on-chip technologies amenable to toxicity testing

M. Shane Hutson, Vanderbilt University

Funded by US EPA

STAR Grant # 83573601

VPROMPT

University of Pittsburgh
Center for
Cellular & Molecular
Engineering

Drug Discovery Institute
UNIVERSITY OF PITTSBURGH

VIBRE
Vanderbilt Institute for Integrative
Biosystems Research & Education

VPROMPT - Vanderbilt-Pittsburgh Resource for Organotypic Models for Predictive Toxicology

PEER-REVIEWED PUBLICATIONS

1. Auner, A.W., Tasneem, K.M., Markov, D.A., McCawley, L.J. and Hutson, M.S., (2019) “Chemical-PDMS Binding Kinetics and Implications for Bioavailability in Microfluidic Devices” *Lab on a Chip* 19: 864-874.
2. Richardson L, Gnecco JS, Ding T, Osteen KG, Aronoff D, Menon R. (2019) “Fetal Membrane Organ-on-Chip: An Innovative Approach to Study Cellular Interactions” *Reproductive Sciences* 2019 Feb 21: 193371911828084 (Epub ahead of print).
3. Gnecco JS, Ding T, Smith C, Lu J, Bruner-Tran KL, Osteen KG (2019) “Hemodynamic Forces Promote the Initiation of Perivascular Decidualization via Endothelial-Derived PGE2 and PGI2 in an Organ-on-Chip Model of the Human Endometrium” *Human Reproduction* 34(4): 702-714.
4. Ding T, Lambert LA, Aronoff DA, Osteen KG, Bruner-Tran KL. (2018) “Sex-Dependent Influence of Developmental Toxicant Exposure on Group B Streptococcus-Mediated Preterm Birth in a Murine Model” *Reproductive Sciences* 25(5):662-673.
5. Bruner-Tran KL, Mokshagundam S, Herington JL, Ding T, Osteen KG (2018) “Rodent Models of Experimental Endometriosis: Identifying Mechanisms of Disease and Therapeutic Targets” *Current Women’s Health Rev* Jun;14(2):173-188.
6. Li X, George SM, Vernetti L, Gough AH, Taylor DL (2018) ”A glass-based, continuously zonated and vascularized human liver acinus microphysiological system (vLAMPS) designed for experimental modeling of diseases and ADME/TOX”, *Lab Chip*. 2018 Aug 21;18(17):2614-2631.
7. Miller DR, McClain ES, Cliffel DE (2018) “Electrochemical Microphysiometry Detects Cellular Glutamate Uptake,” J. *Electrochem. Soc*. 2018 Aug. 165: G3120-G3124.
8. Lee-Montiel FT, George SM, Gough AH, Sharma AD, Wu J, DeBiasio R, Vernetti LA, Taylor DL (2017) “Control of oxygen tension recapitulates zone-specific functions in human liver microphysiology systems”, *Experimental Biology in Medicine* 2017 Oct;242(16):1617-1632.

PEER-REVIEWED PUBLICATIONS

9. Karolak A, Markov DA, McCawley LJ and Rejniak KA (2017) “Towards personalized computational oncology: from spatial models of tumour spheroids, to organoids, to tissues” *J. R. Soc. Interface* 15, 2018, pp 20170703.

10. Cyr KJ, Avaldi OM, Wikswo JP (2017) “Circadian Hormone Control in a Human-on-a-Chip: In Vitro Biology’s Ignored Component?” *Exp. Biol. Med.* 2017 Nov. 242(17):1714-1731.

11. Watson DE, Hunziker R, Wikswo, JP (2017) “Fitting Tissue Chips and Microphysiological Systems into the Grand Scheme of Medicine, Biology, Pharmacology, and Toxicology” *Exp. Biol. Med.* 2017 Oct. 242(16): 1559-1572.

12. Bruner-Tran KL, Gnecco, JS, Ding T, Glore DR, Pensabene V, Osteen KG (2017) “Exposure to the Environmental Endocrine Disruptor TCDD and Human Reproductive Dysfunction: Translating Lessons from Murine Models” *Reprod Toxicol.* 2017 Mar;68:59-71.

13. Gnecco JS, Pensabene V, Li D, Ding T, Hui E, Bruner-Tran KL, Osteen KG (2017) “Compartmentalized culture of perivascular stroma and endothelial cells in amicrofluidic model of the human endometrium” *Ann Biomed Eng* (2017) 45: 1758.

14. Gnecco JS, Anders AP, Cliffel D, Pensabene V, Osteen KG, Aronoff DM. (2017) “Instrumenting a Fetal Membrane on a Chip as Emerging Technology for Preterm Birth Research”. *Current Pharmaceutical Design*, 23 (46). ISSN 1381-6128

15. Soto-Gutierrez A, Gough A, Vernetti LA, Taylor DL, Monga SP (2017) “Pre-Clinical and Clinical Investigations of Metabolic Zonation in Liver Diseases: The Potential of Microphysiology Systems” *Experimental Biology and Medicine* 2017; 0: 1-12.

16. Vernetti LA, Vogt A, Gough A, Taylor DL (2017) Book Chapter: "Evolution of Experimental Models of the Liver to Predict Human Drug Hepatotoxicity and Efficacy". *Clinics in Liver Disease*, February 2017, 21: 197-214.

PEER-REVIEWED PUBLICATIONS

17. Vernetti LA, Gough A, Baetz N, Blutt S, Broughman JR, Brown JA, Foulke-Abel J, Hasan N, In J, Kelly E, Kovbasnjuk O, Repper J, Senutovitch N, Stabb J, Yeung C, Zachos NC, Donowitz M, Estes M, Himmelfarb J, Truskey G, Wikswo JP, Taylor DL (2017) "Functional Coupling of Human Microphysiology Systems: Intestine, Liver, Kidney Proximal Tubule, Blood-Brain Barrier and Skeletal Muscle", *Scientific Reports* 7: 42296, Feb 2017.

18. Hutson MS, Leung MCK, Baker NC, Spencer RM, Knudsen TB (2017) "Computational Model of Secondary Palate Fusion and Disruption", *Chem. Res. Toxicol.*, Jan 2017.

19. Dodds JN, May JC, McLean JA (2016) "Investigation of the Complete Suite of the Leucine and Isoleucine Isomers: Toward Prediction of Ion Mobility Separation Capabilities", *Anal. Chem.* 89, 952−959, Dec 2016.

20. Alexander PG, Clark KL, Tuan RS (2016) "Prenatal Exposure to Environmental Factors and Congenital Limb Defects", *Birth Defects Res (Part C)* 108:243-273, 2016.

21. M.S. Hutson, P.G. Alexander, V. Allwardt, D.M. Aronoff, K.L. Bruner-Tran, D.E. Cliffel, J.M. Davidson, A. Gough, D.A. Markov, L.J. McCawley, J.R. McKenzie, J.A. McLean, K.G. Osteen, V. Pensabene, P.C. Samson, N.K. Senutovitch, S.D. Sherrod, M.S. Shotwell, D.L. Taylor, L.M. Tetz, R.S. Tuan, L.A. Vernetti and J.P. Wikswo (2016) "Organs-on-Chips as Bridges for Predictive Toxicology" *Applied In Vitro Toxicology* 2(2): 97-102.

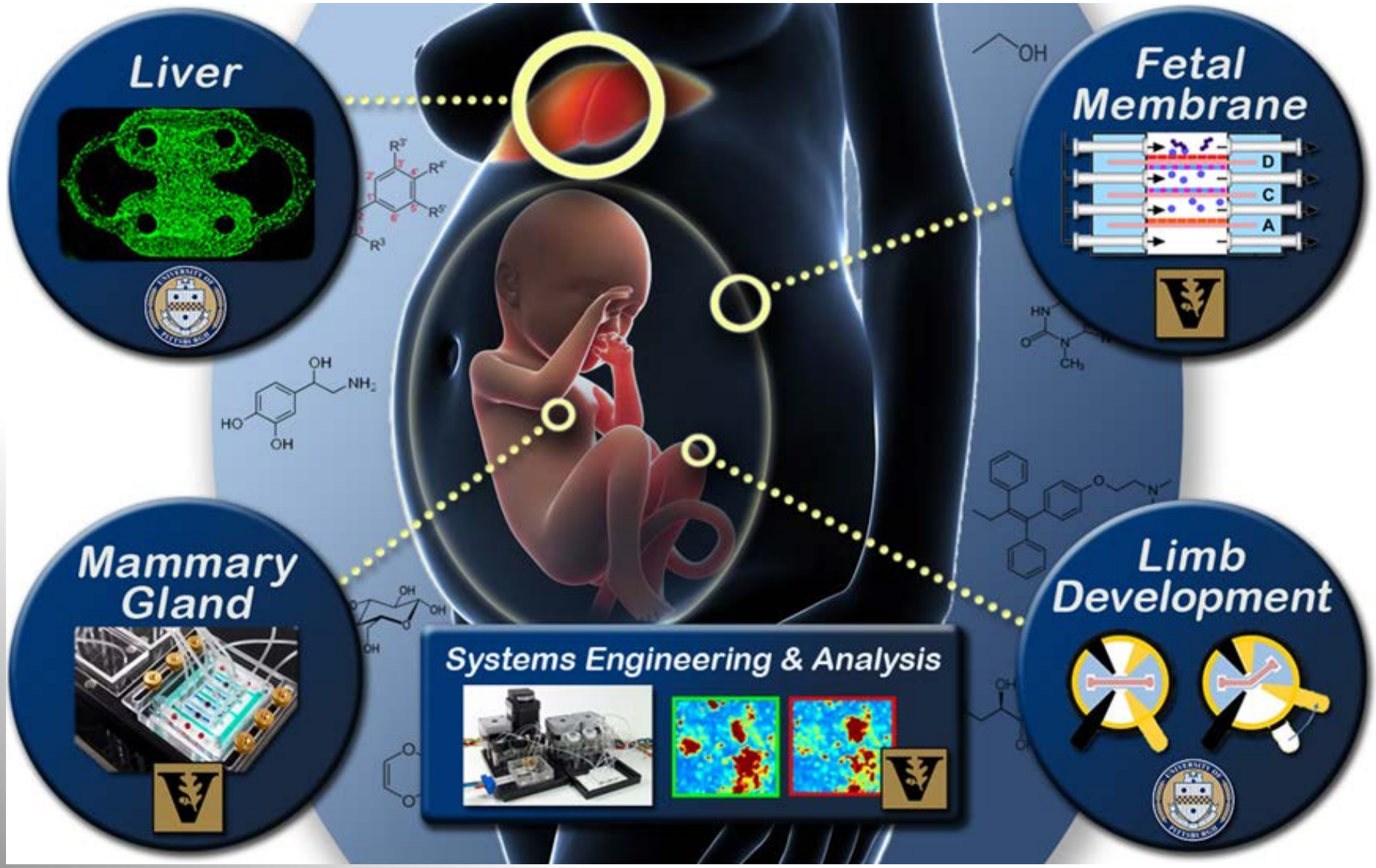
Plus …. 4 patent applications (and counting)

an R&D 100 Award for Co-PI Wikswo and collaborators

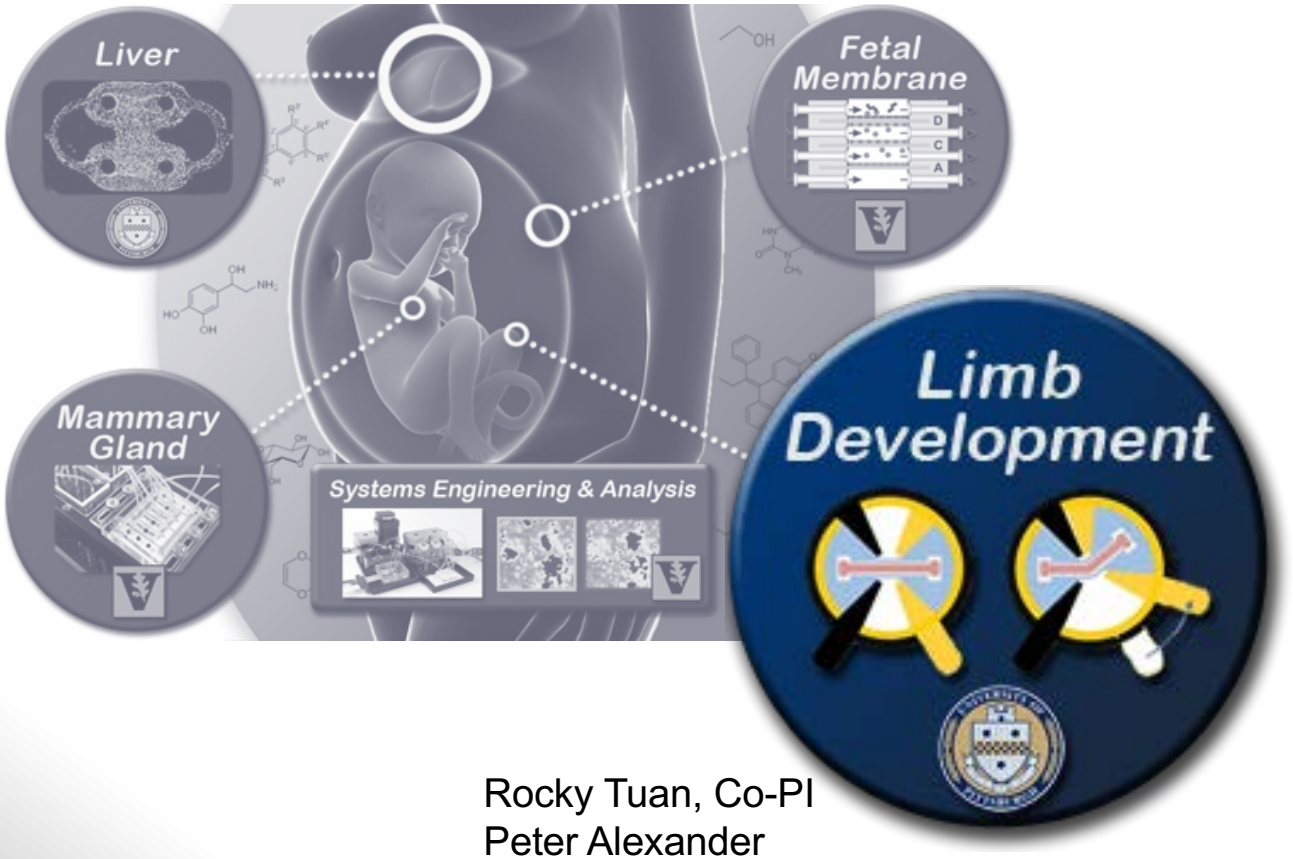
student awards at the Teratology Conference and annual meeting of AIChE

51 talks (and counting) at universities and scientific conferences

VPROMPT: **V**anderbilt-**P**ittsburgh **R**esource for **O**rganotypic **M**odels for **P**redictive **T**oxicology



PROJECT 2 – LIMB DEVELOPMENT

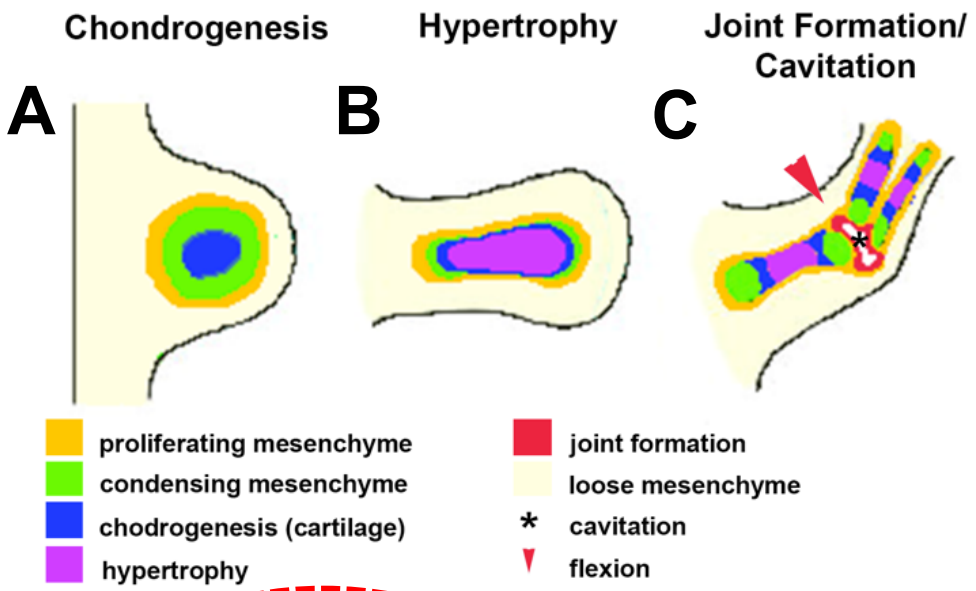


Rocky Tuan, Co-PI
Peter Alexander
Karen Clark

10/28/19

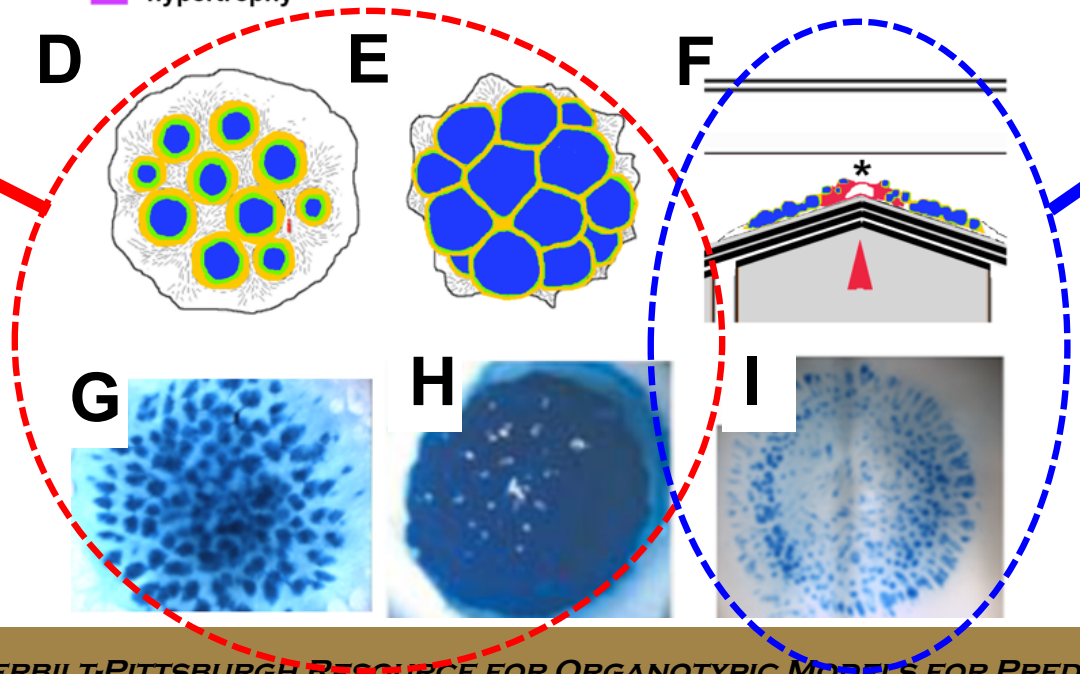
LIMB DEVELOPMENT

SUMMARY OF MODELS



OCM 1 & 2

OCM = Organotypic Culture Model



OCM 3

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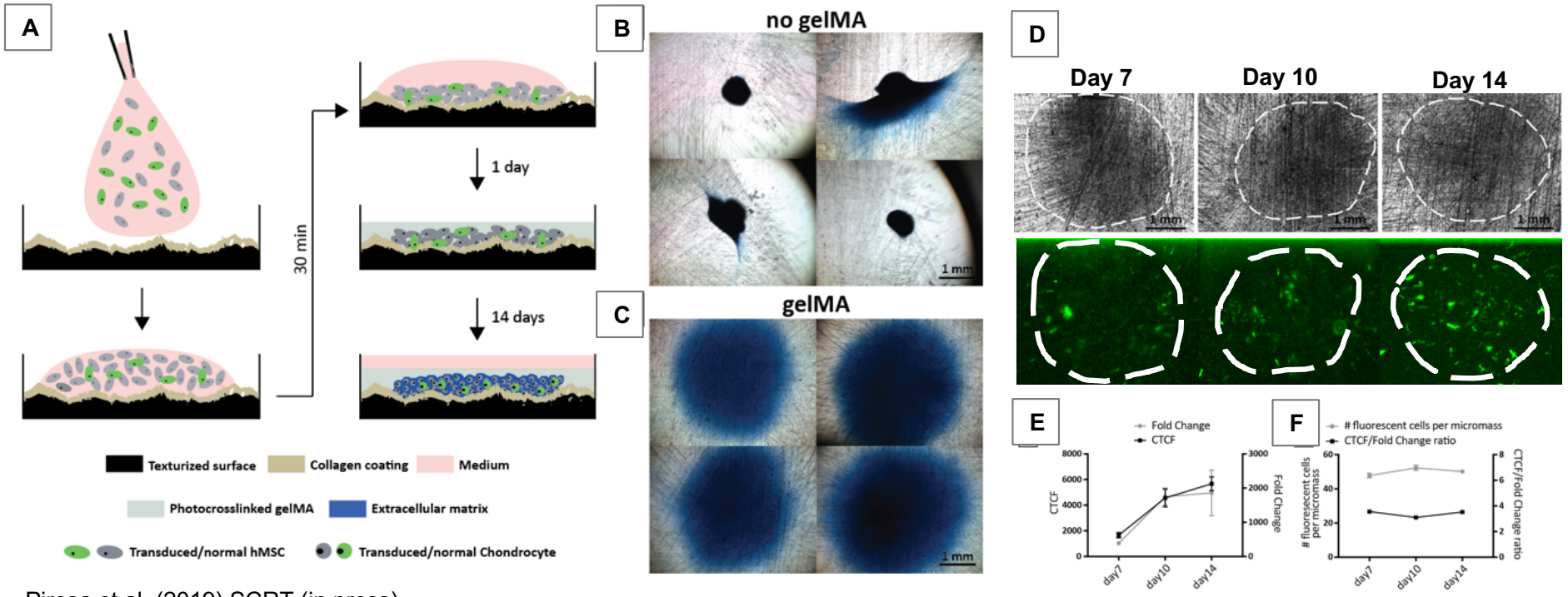
LIMB DEVELOPMENT

1. Development of a small, hMSC-based high density micromass culture with uniform morphology was developed.



Pirosa et al. (2019) SCRT (in press)

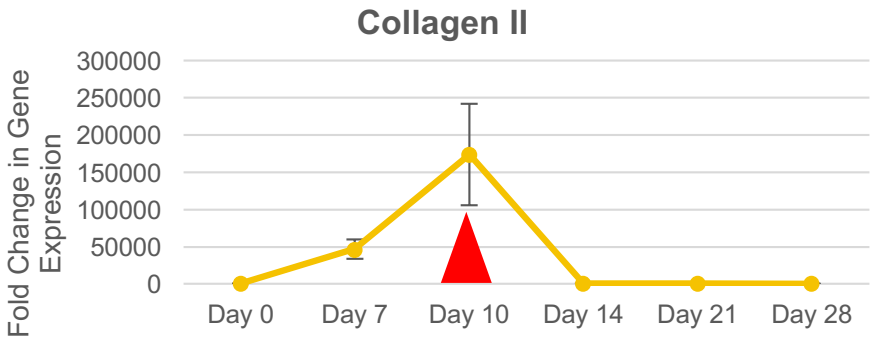
2. And validation of chondrogenic and hypertrophic GFP-promoter-reporter constructs were validated – COLII shown

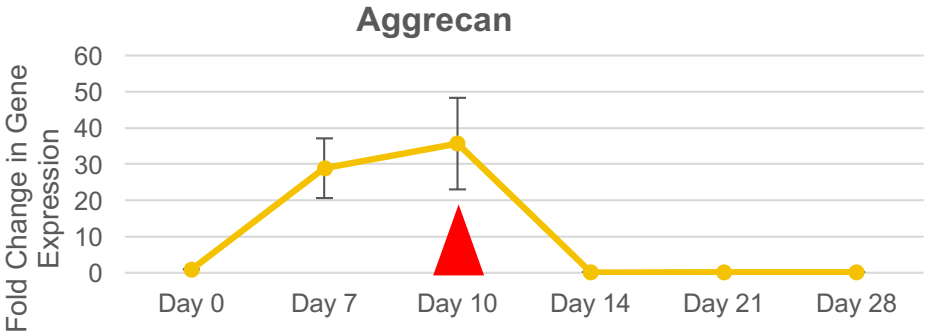
LIMB DEVELOPMENT

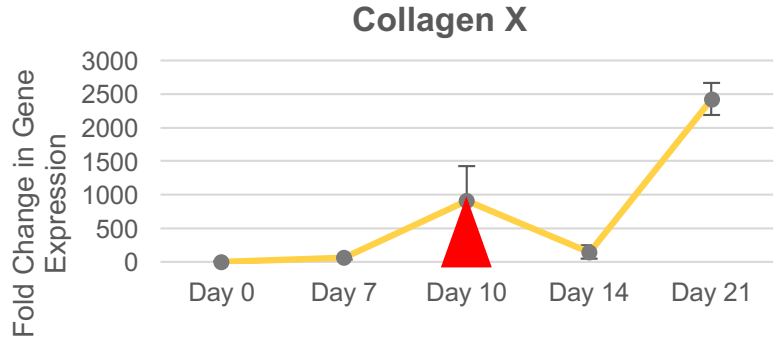
3. hMSC cultures undergo T3-induced hypertrophy

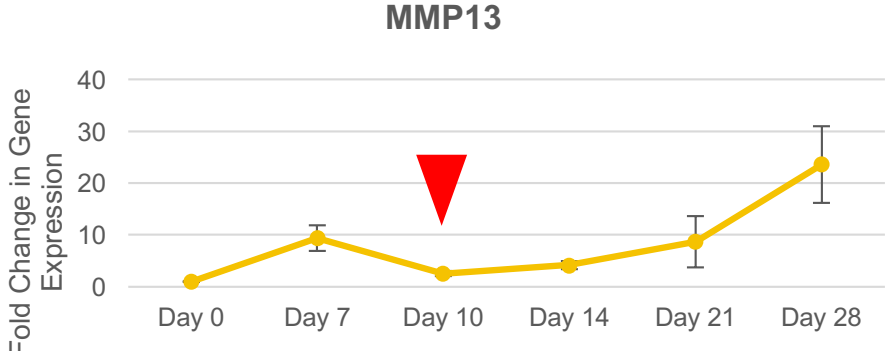
Chondrogenic

Hypertrophic

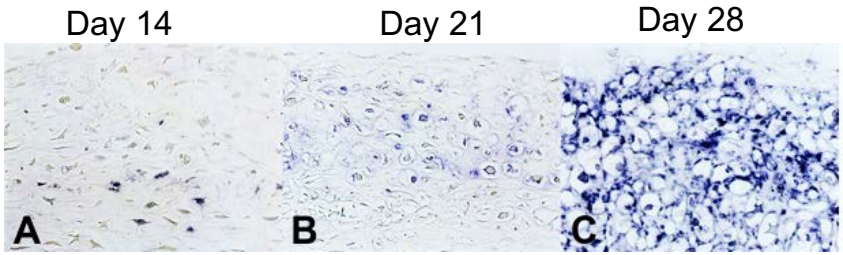








Alkaline Phosphatase Histochemistry



10/28/19

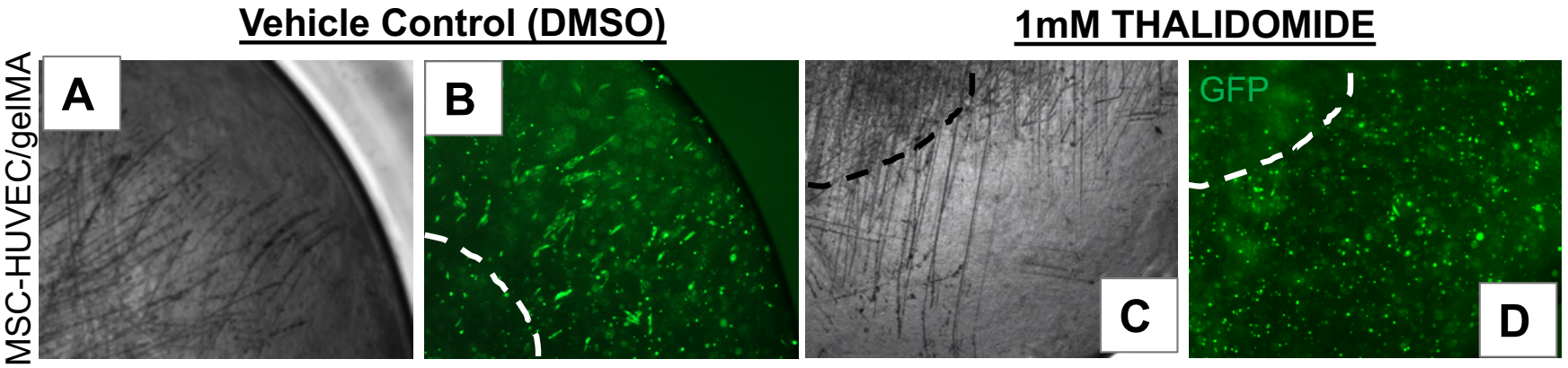
LIMB DEVELOPMENT

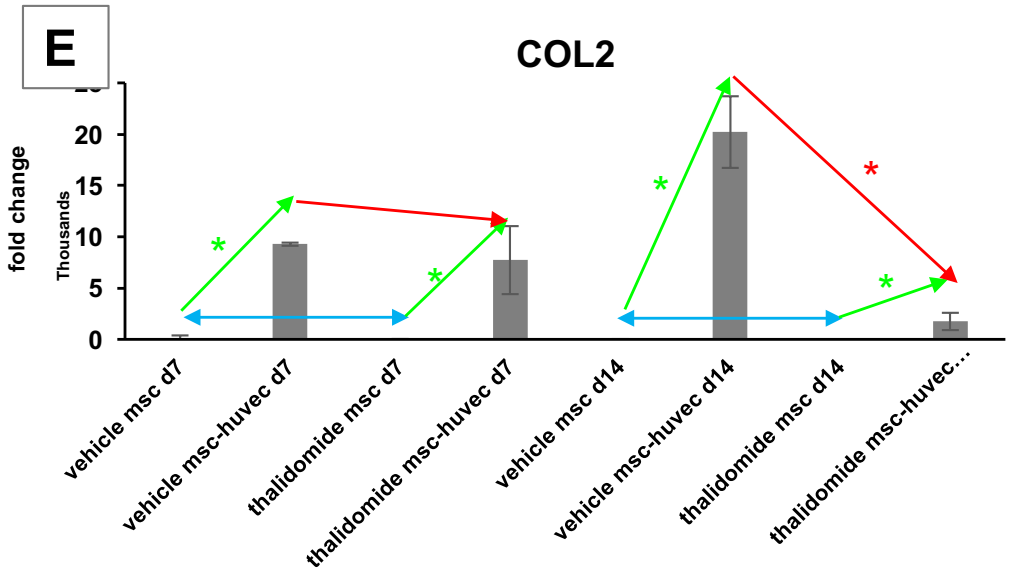
4. hMSC micromass cultures respond to <u>three (3) known</u> teratogens in a stage-specific manner

- **Valproic Acid (VPA):** 1-10 μM. Chondrogenesis was more sensitive to VPA treatment than hypertrophy, a difference that may be related to the VPA mechanism of action of HDAC activity and potential inhibition of mesenchymal differentiation.
- **Warfarin**: 100 nM. A known inhibitor of vitamin K-dependent post-translational γ-glutamyl carboxylation of cartilage extracellular matrix protein involved in mineralization. Hypertrophic cultures were more sensitive than chondrogenic cultures, likely due to the requirement of matrix mineralization during late cartilage hypertrophy.
- **Thalidomide**. <u>*No effect observed on either chondrogenesis or hypertrophy!*</u> Thalidomide is recently described as an inhibitor of cereblon, a ubiquitin ligase substrate adapter protein, that results in reduced turnover of a subset of proteins that results in reduced growth and cell death, and likely to affect skeletal development via targets other than chondrogenesis and hypertrophy (likely targeting angiogenesis).

LIMB DEVELOPMENT

5. Addition of HUVECs in gelMA overlay results in a thalidomide-sensitive hMSC micromass culture





In MSC-based chondrogenic micromass cultures, thalidomide has no significant effect on gene expression

The addition of HUVECs to the culture increases MSC chondrogenesis

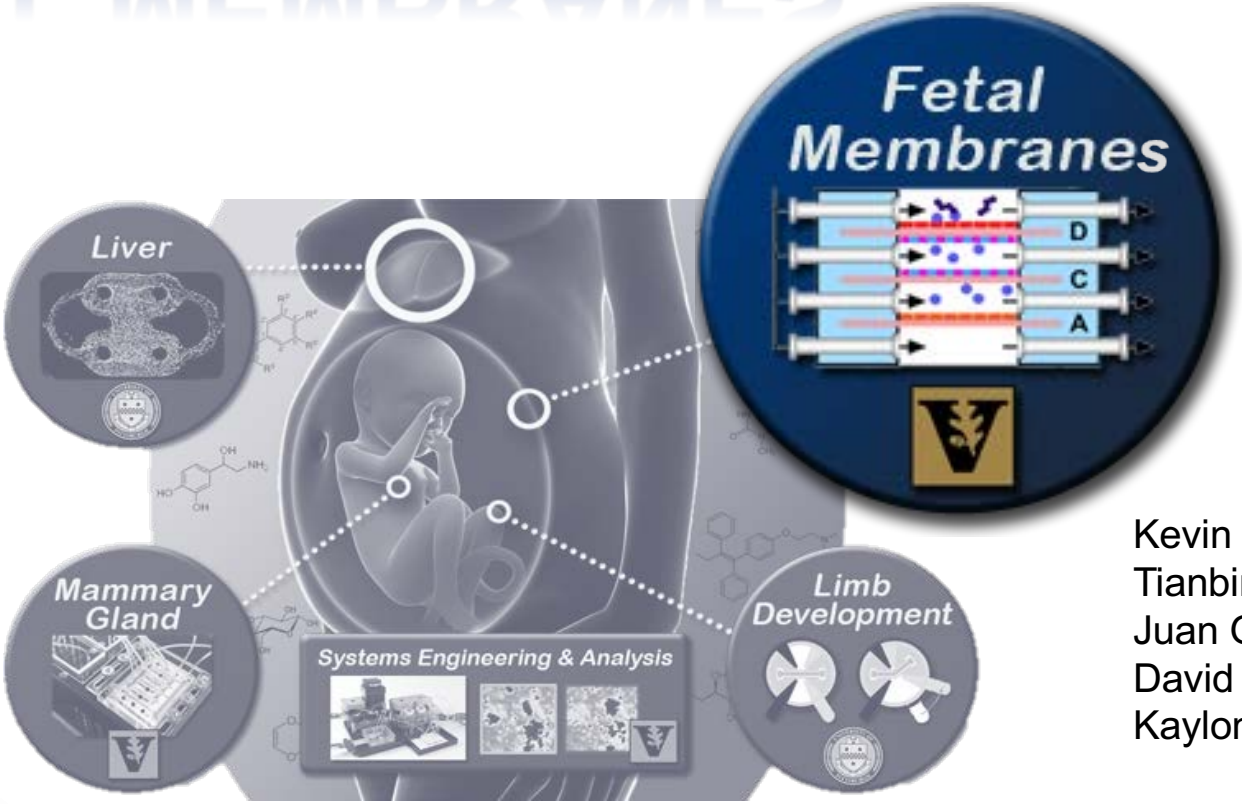
Thalidomide causes a decrease in MSC chondrogenic gene expression.

LESSON #1

OCMs for use in toxicity testing need to be fit-for-purpose.

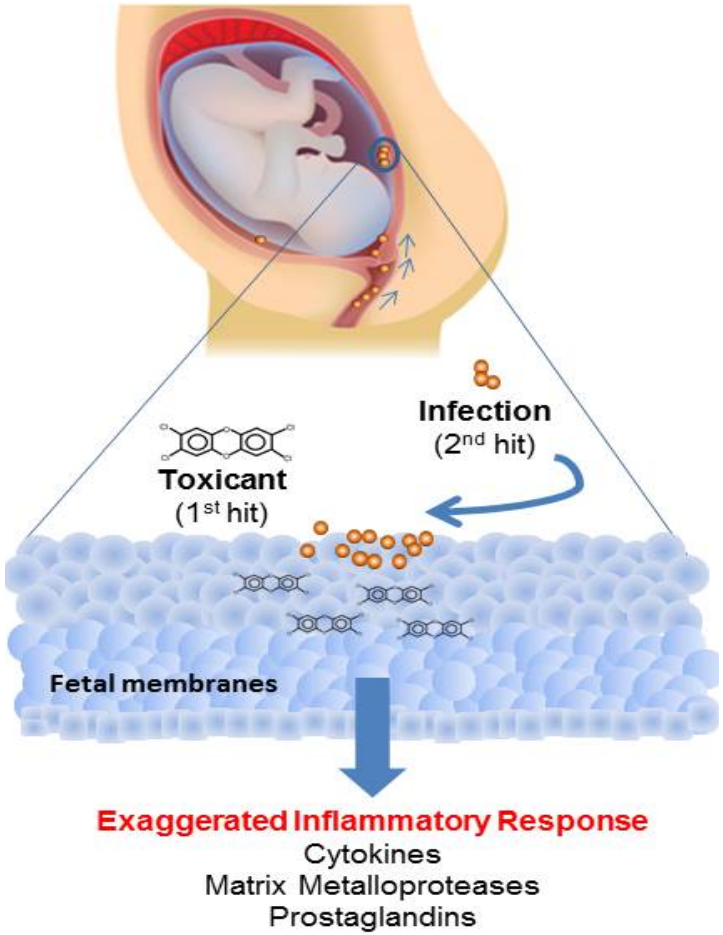
They should be as complex as necessary, but no more so.

PROJECT 3 – FETAL MEMBRANES



Kevin Osteen, Co-PI
Tianbing Ding
Juan Gnecco
David Aronoff
Kaylon Bruner-Tran

PREGNANCY RELATED COMPLICATIONS: PRETERM BIRTH



Preterm birth (PTB) is the leading cause of child mortality

Chorioamnionitis (CAM), or intrauterine infection during pregnancy, is a leading cause of PTB.

However, not all women with microbial contamination of the amniotic cavity deliver preterm, suggesting host factors influence risk for CAM-associated PTB.

Hypothesis: environmental toxicant exposure primes the gravid uterus for an exaggerated inflammatory responses to microbial invasion.

Rats exposed to TCDD as pups exhibit an endometriosis-like phenotype and higher rates of PTB later in life: Bruner-Tran and Osteen 2011.

Air Pollution from Incinerators and Reproductive Outcomes

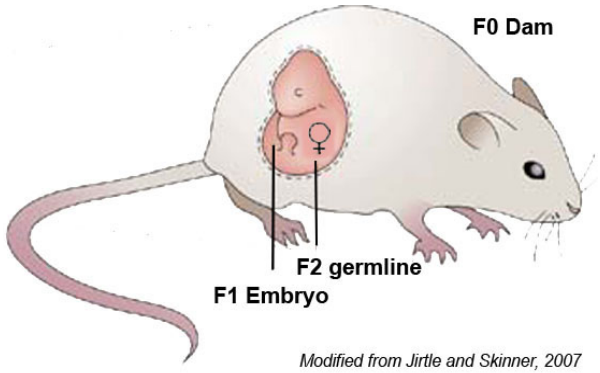
A Multisite Study

Silvia Candela,[a] Andrea Ranzi,[b] Laura Bonvicini,[a] Flavia Baldacchini,[a] Paolo Marzaroli,[a] Andrea Evangelista,[a] Ferdinando Luberto,[a] Elisa Carretta,[a] Paola Angelini,[c] Anna Freni Sterrantino,[b] Serena Broccoli,[a] Michele Cordioli,[b] Carla Ancona,[d] and Francesco Forastiere[d]

Human epidemiology studies report an association between endometriosis and PTB: Brosens et al, 2015; Stern et al, 2015; Vigano et al, 2015; Exacoustos et al, 2016.

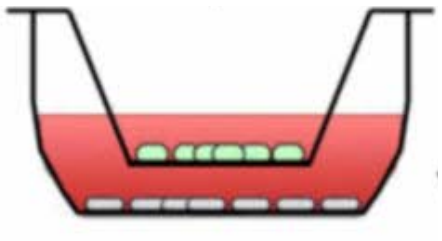
MODELING PREGNANCY-ASSOCIATED EVENTS

Mouse developmental exposure model

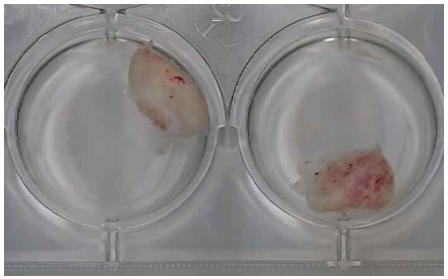


Modified from Jirtle and Skinner, 2007

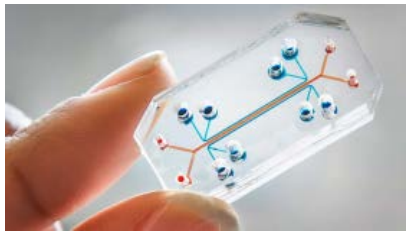
Human tissue/cell culture models



Static cell cultures

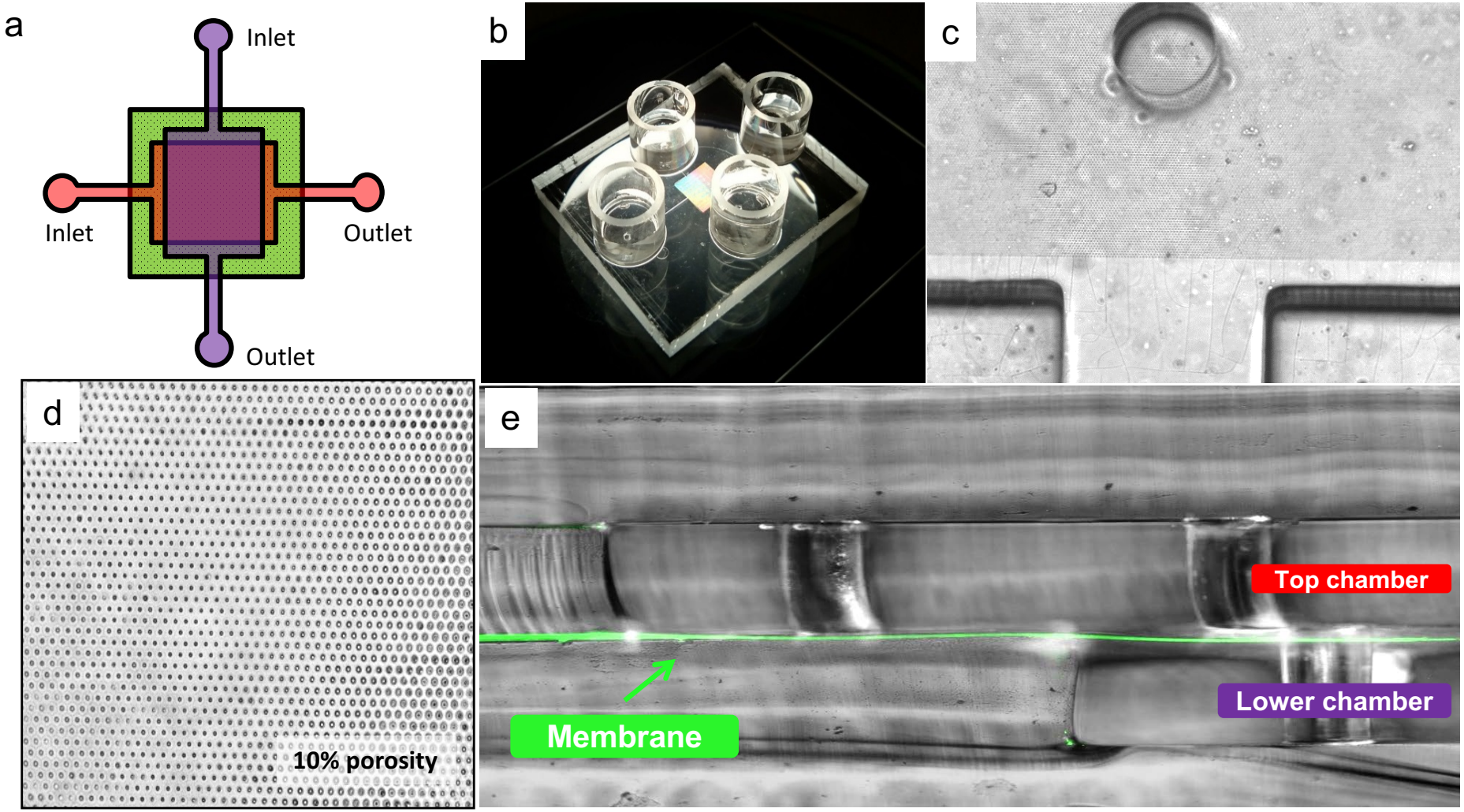


Punch biopsies/organ culture

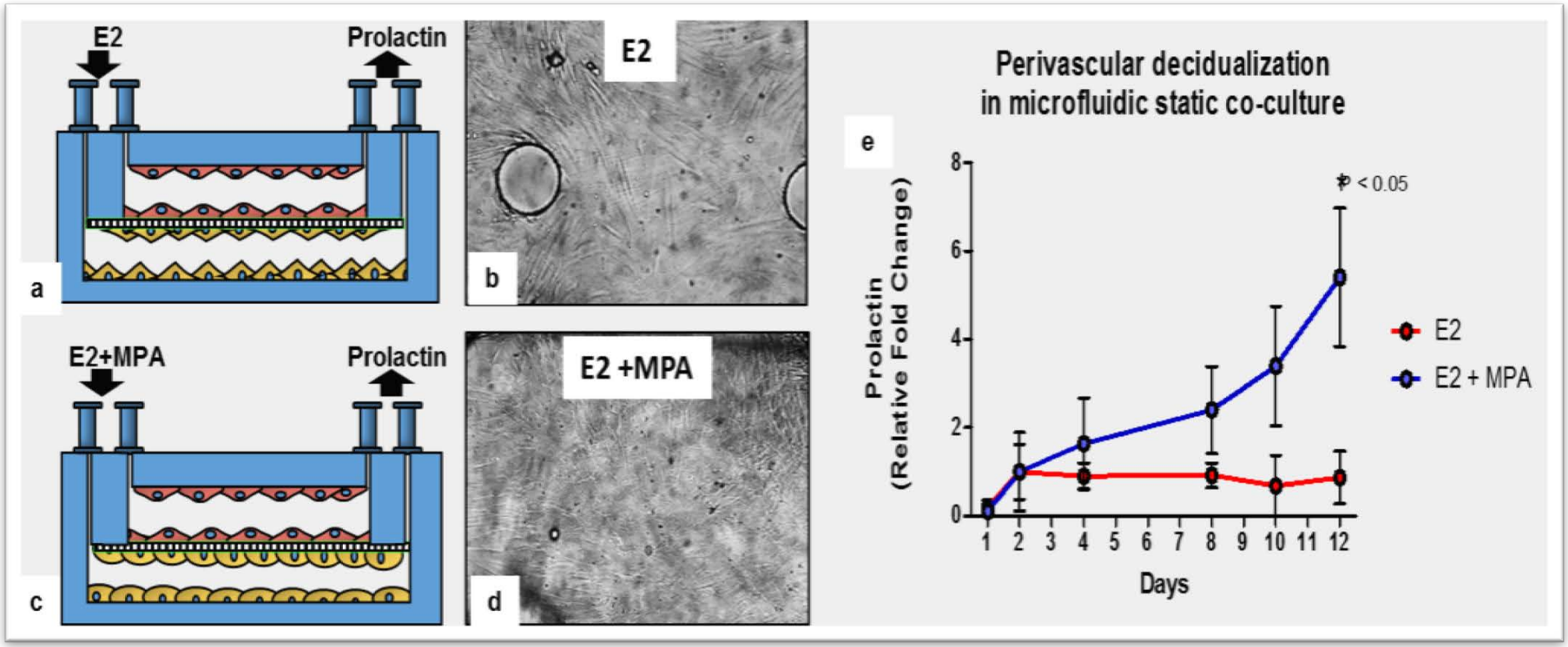


Organ on Chip

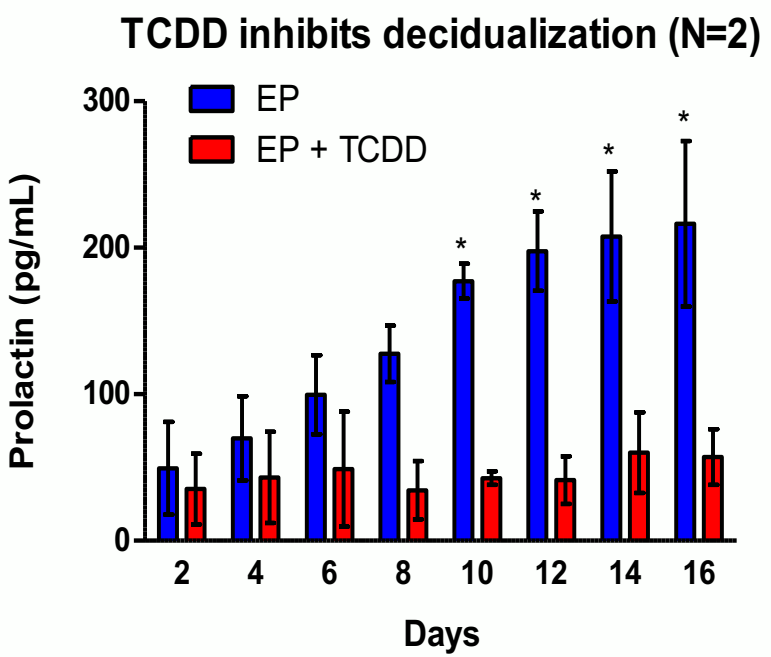
DESIGN AND CHARACTERIZATION OF THE DUAL CHAMBER MICROFLUIDIC DEVICE



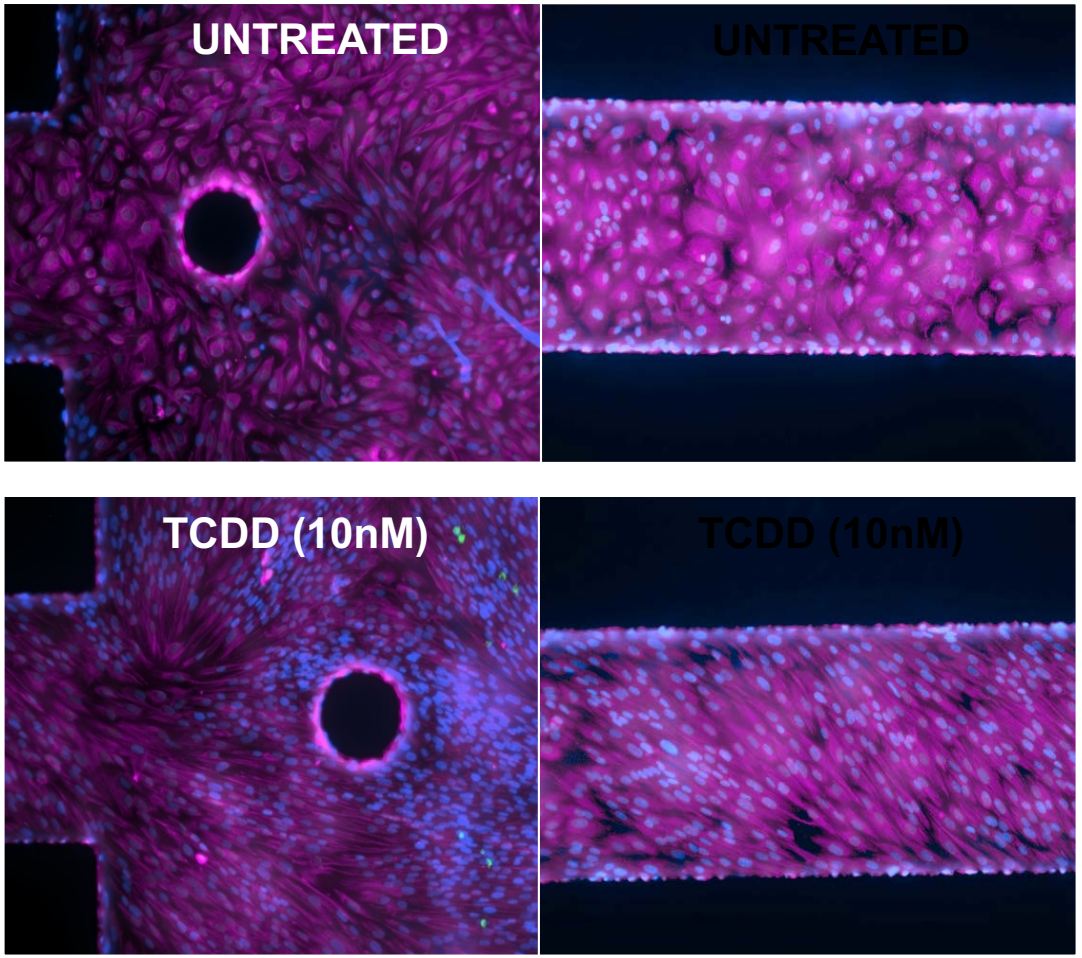
DECIDUALIZATION WITHIN DUAL CHAMBERED DEVICE



IN-DEVICE TCDD EXPOSURE IMPAIRS MORPHOLOGICAL AND BIOCHEMICAL MARKERS OF PROGESTIN ACTION



For more details, see Kevin Osteen's talk this afternoon.



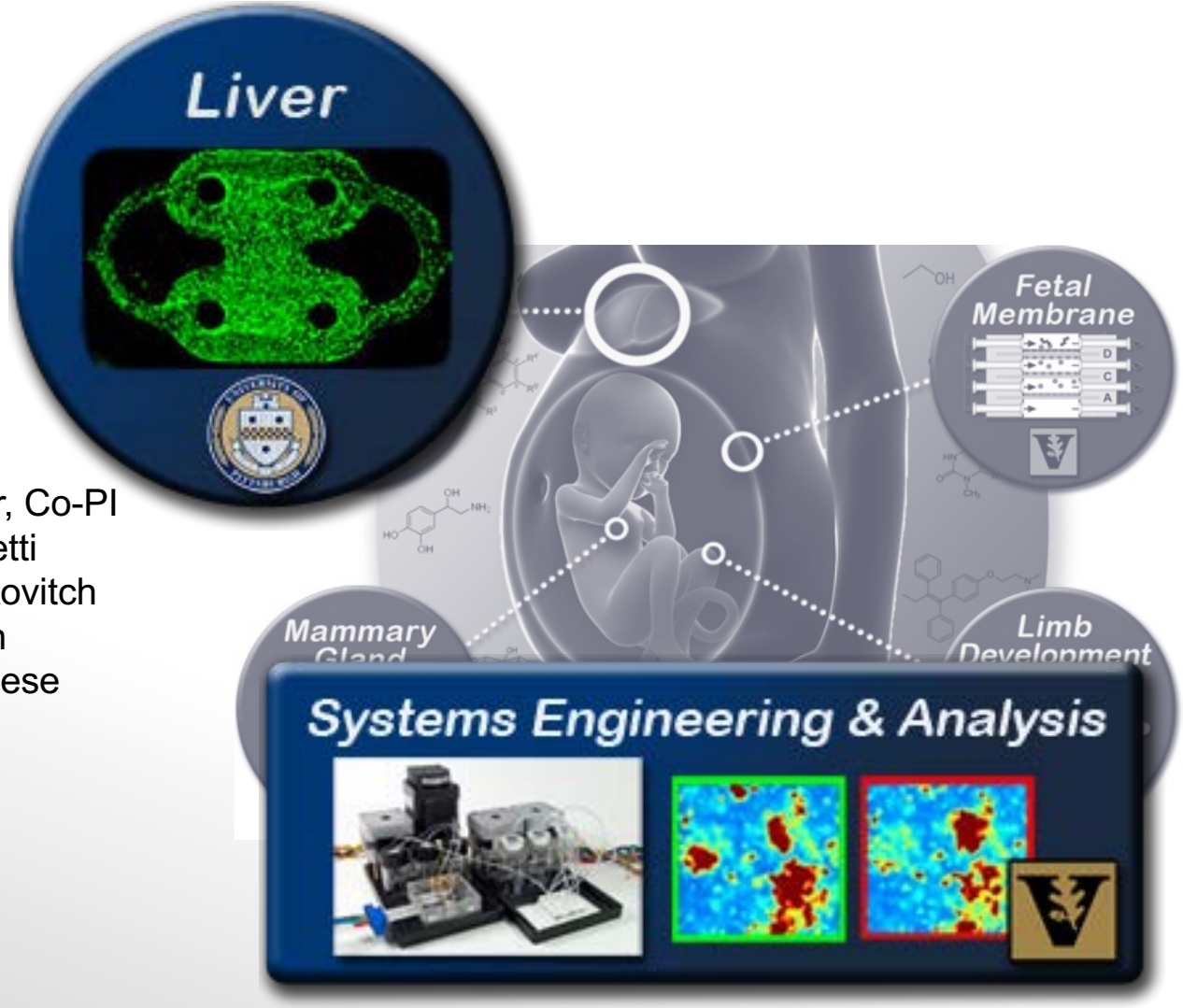
Vimentin, DAPI

LESSON #2

Building and testing an OCM really is a test of the plausibility and completeness of the underlying AOP (Adverse Outcome Pathway).

Should couple the OCM and AOP explicitly.

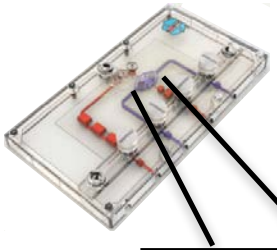
PROJECT 4 – LIVER



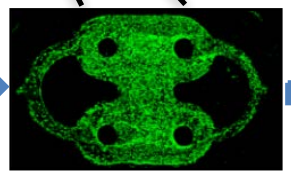
Lans Taylor, Co-PI
Larry Vernetti
Nina Senutovitch
Bert Gough
Celeste Reese

The 4 Human Cell Type, 3D Liver Organotypic Culture Model (OCM) is a Component of the UPitt Integrated Platform for Predictive Toxicology

A **Human 4 cell Liver Acinus Microphysiology System Model**



Cells
- Hepatocytes
- Endothelial cells
- Kupffer cells
- Stellate cells



Pump

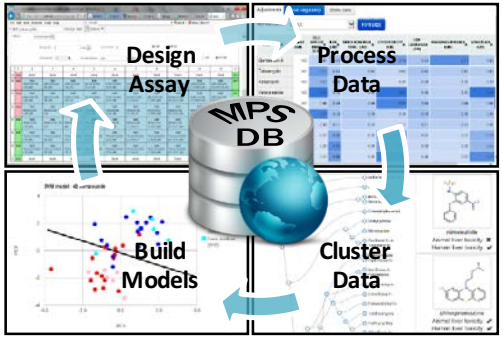
Influx Media

Efflux Media

D Scaling Factors to Integrate Liver OCM
1 Liver OCM: 3 Mammary OCM
1 Liver OCM: 2 Fetal Membrane OCM
1 Liver OCM: 1 Limb Bud OCM

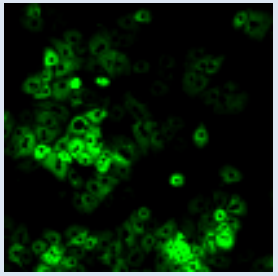
C **Media Measurements**
Albumin, Urea, LDH
Drug Metabolism

E **MicroPhysiology Database**

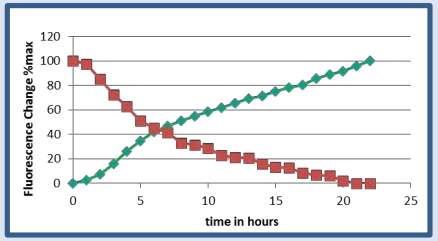


B **High Content Imaging**

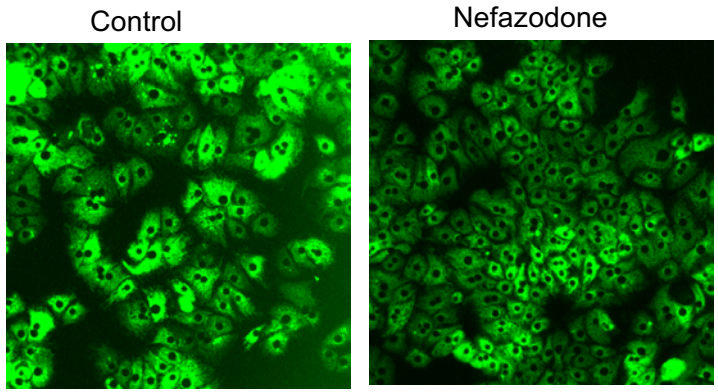
Sentinel Cells

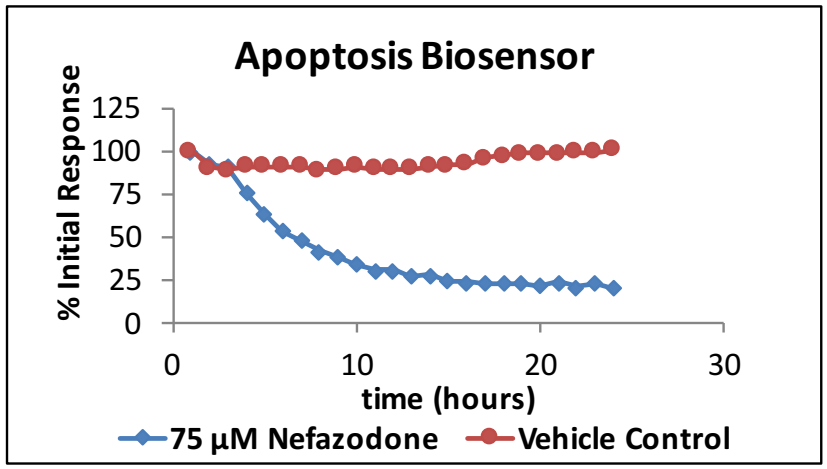


Functional Analysis



Functional Biosensors Developed at UPitt have been Integrated into all of the VPROMPT OCMs for Live Cell Monitoring of Toxicological Pathways





Organotypic Model & Cells	Project Biosensor(s)
Project 1: Mammosphere *1st-MCF-7, MCF10A* *2nd-primary mammary epithelial cells, fibroblasts, subcutaneous adipocytes*	Proliferation pCT-H2B-GFP Apoptosis pCT-mito-GFP
Project 2: Limb Bud *1st-Rat* *2nd-human mesenchymal stem cells*	Proliferation/Cell tracking pCT-H2B-GFP Apoptosis pCT-mito-GFP
Project 3: Fetal Membrane *1st-mouse amniotic epithelial cells, mesenchymal fibroblasts, chorionic tropoblasts, decidual cells, THP-1* *2nd-human primaries*	Proliferation/Cell tracking pCT-H2B-GFP
Project 4: Liver *Hepatocytes, stellate cells*	Apoptosis pCT-mito-GFP pCT-mito-mKate2

10/28/19

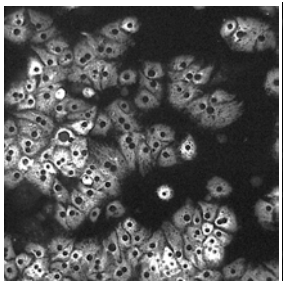
Multiplexing: 1 OCM, 29 Assays

Assay & Sampling Collection Points

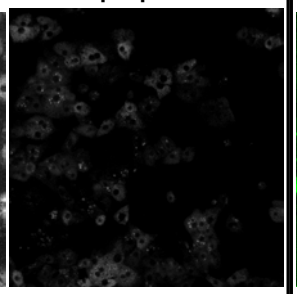
Assay/Sample Day	In Life Sampling Collection Points (days)																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Image Collection																		
Apoptosis Biosensor[1]					X													X
Bile Canalicular Efflux[2]					X													
Mass Spec Analytical																		
Cyp 3A4 induction						X												
Media Secretion Assays																		
Albumin					X						X						X	
Urea					X						X						X	
LDH	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
TNF-α		X																

1. Apoptosis biosensor mitokate2-cytochrome C[1]

Control

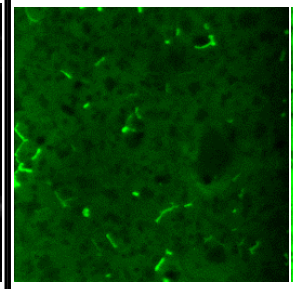


Apoptosis

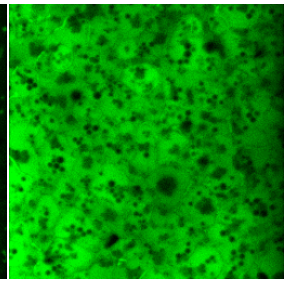


2. Bile Efflux CMFDA dye[1]

Control

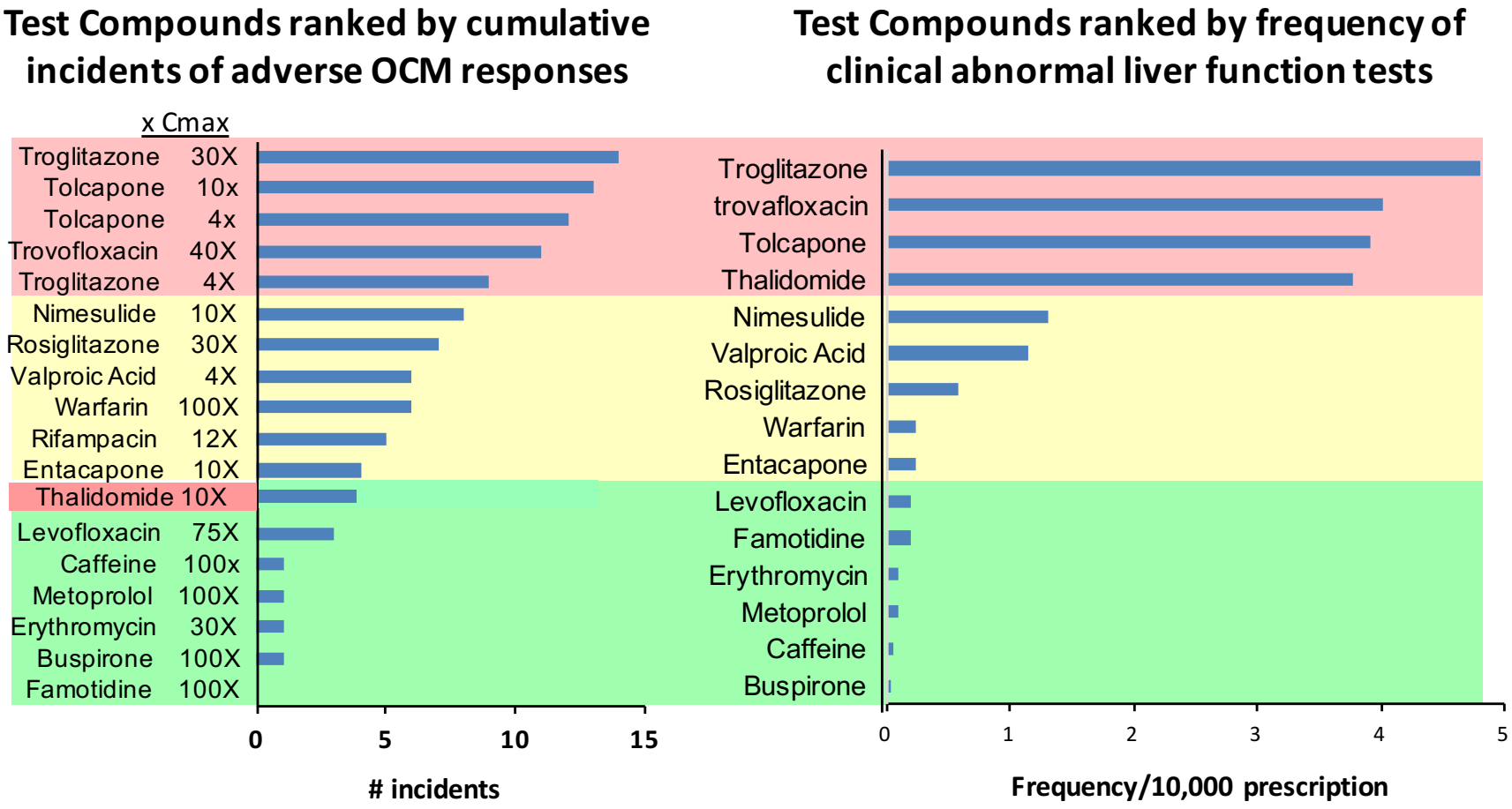


Inhibited



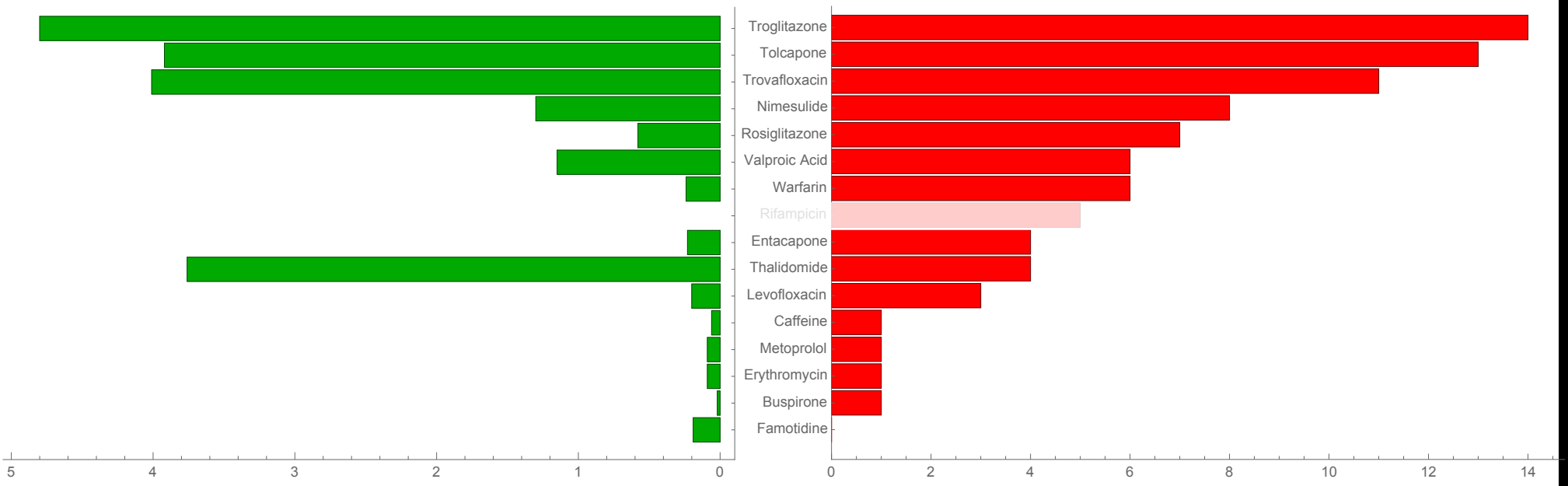
10/28/19

Rank-Ordering of 16 Compounds for Hepatotoxicity in an 18 Day Study in the Liver OCM is concordant with the Normalized Adverse Event Frequency from the MPS-Database*



* Data from the MPS-Database in which the FDA FAERS data is normalized to drug use frequency from the CDC

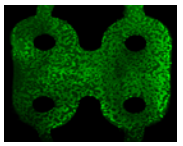
REPLOTTED TO FACILITATE CHEMICAL-BY-CHEMICAL COMPARISONS



Frequency of Adverse Clinical Events (per 10,000 Rx)

$\beta = 0.816$

Max NARLOCM



(NARLOCM = Number of Adverse Responses in Liver OCM)

10/28/19

LESSON #3

Take advantage of multiplexing: assaying multiple endpoints or outputs for a single OCM improves the robustness of its toxicity predictions.

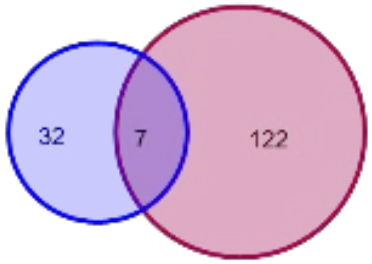
Deep Dives into AOPs using Metabolomics via Ion Mobility Mass Spectroscopy

Statistically significant compounds observed in response to *Tolcapone* and *Entacapone* exposure

Tolcapone (stimulation for 24h)

Entacapone (stimulation for 24h)

The metabolomics signature response to *tolcapone* treatment for 24h



The metabolomics signature response to *entacapone* treatment for 24h

Pathways Enriched

Tolcapone

HISTIDINE METABOLISM
PROPANOATE METABOLISM
PROTEIN BIOSYNTHESIS
VALINE, LEUCINE AND ISOLEUCINE DEGRADATION

Entacapone

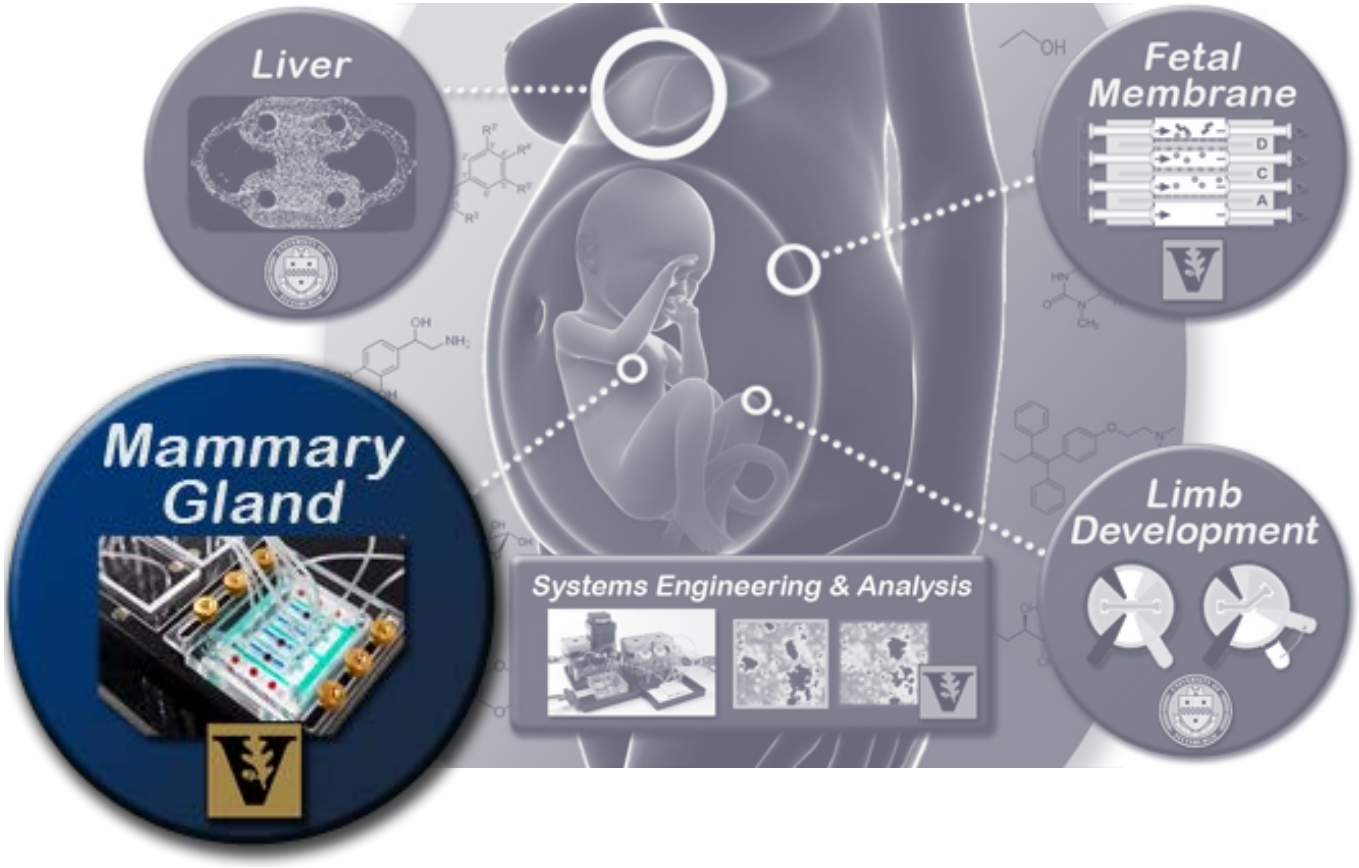
ASPARTATE METABOLISM
CITRIC ACID CYCLE
FRUCTOSE AND MANNOSE DEGRADATION
GALACTOSE METABOLISM
MITOCHONDRIAL ELECTRON TRANSPORT CHAIN
PHENYLALANINE AND TYROSINE METABOLISM
TYROSINE METABOLISM

10/28/19

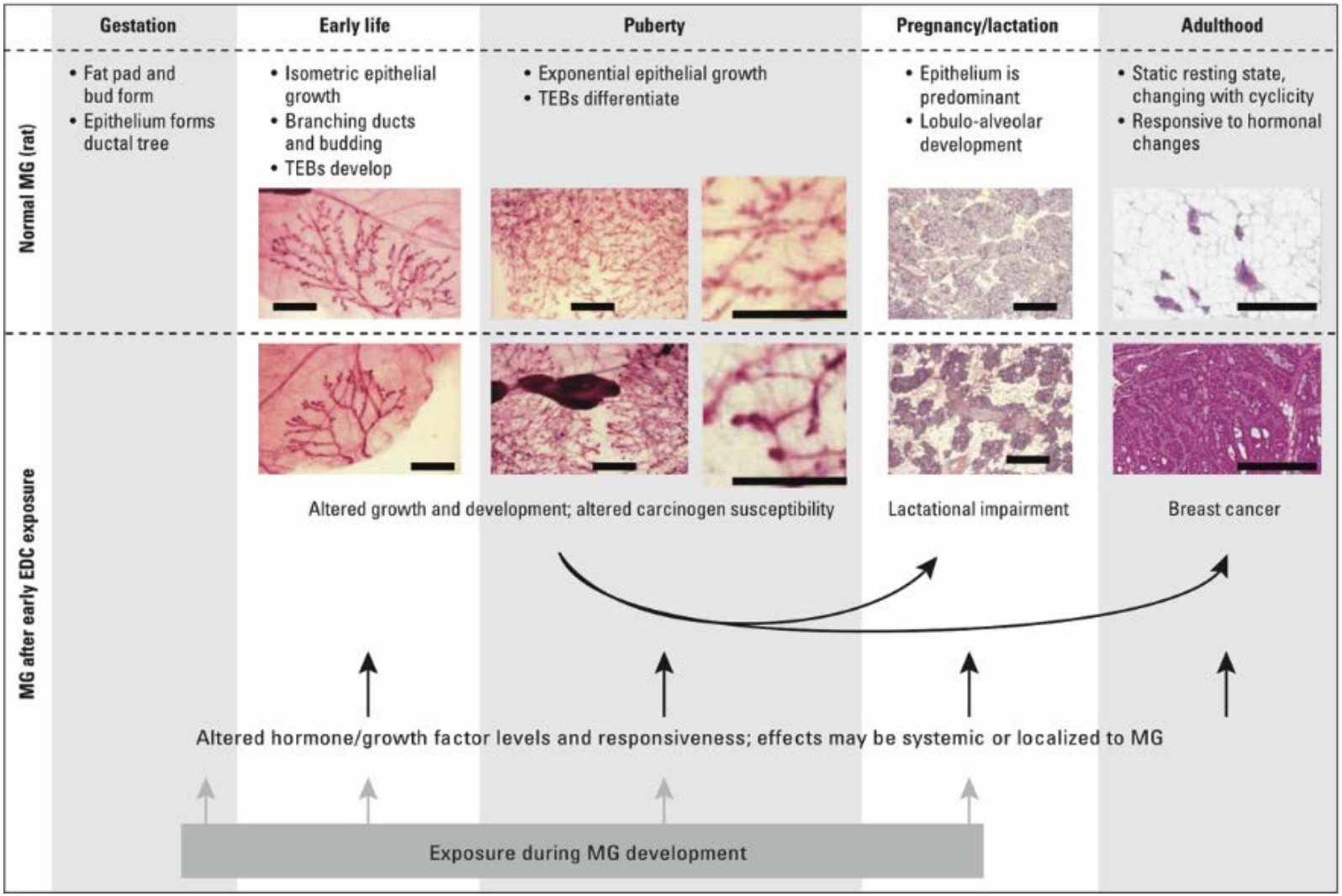
LESSON #4

High to medium throughput screening is not the only use of OCMs; they can also be useful for taking a deep dive into ambiguous portions of their coupled AOPs.

PROJECT 1 – MAMMARY GLAND

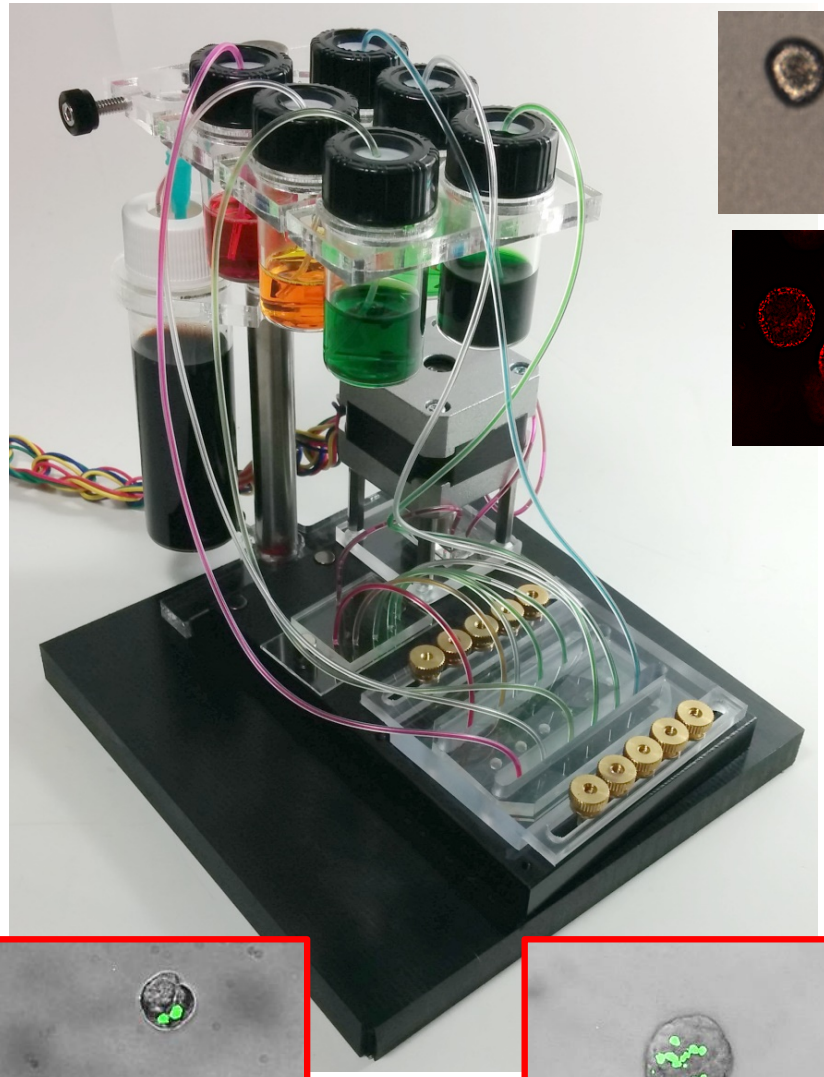


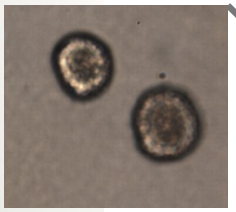
Mammary Gland development and effects of environment on subsequent events

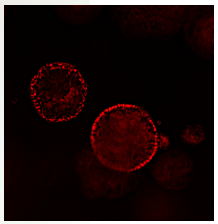


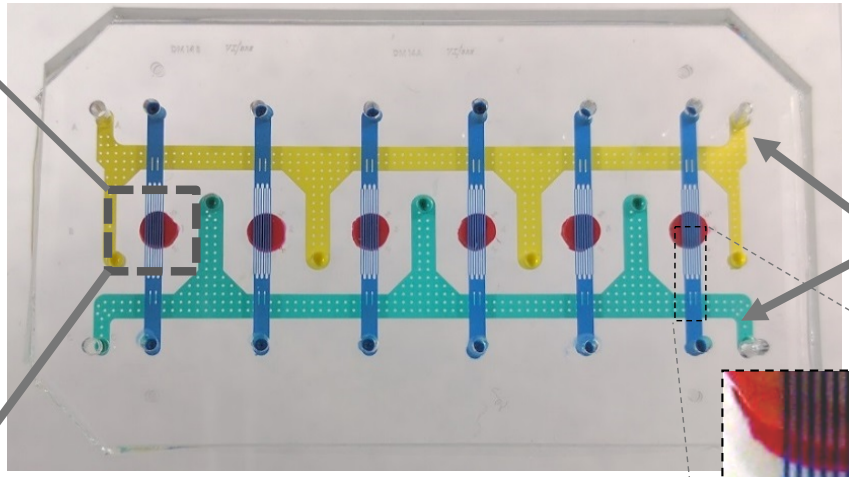
Rudel et al, Environ Health Perspect 119:1053-1061 (2011).

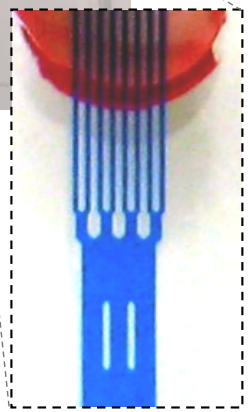
Thick Tissue Bioreactor for long-term mammosphere culture

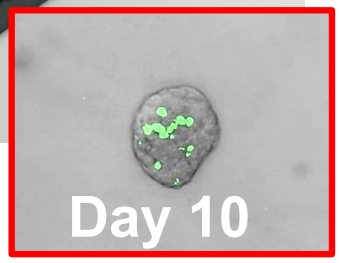








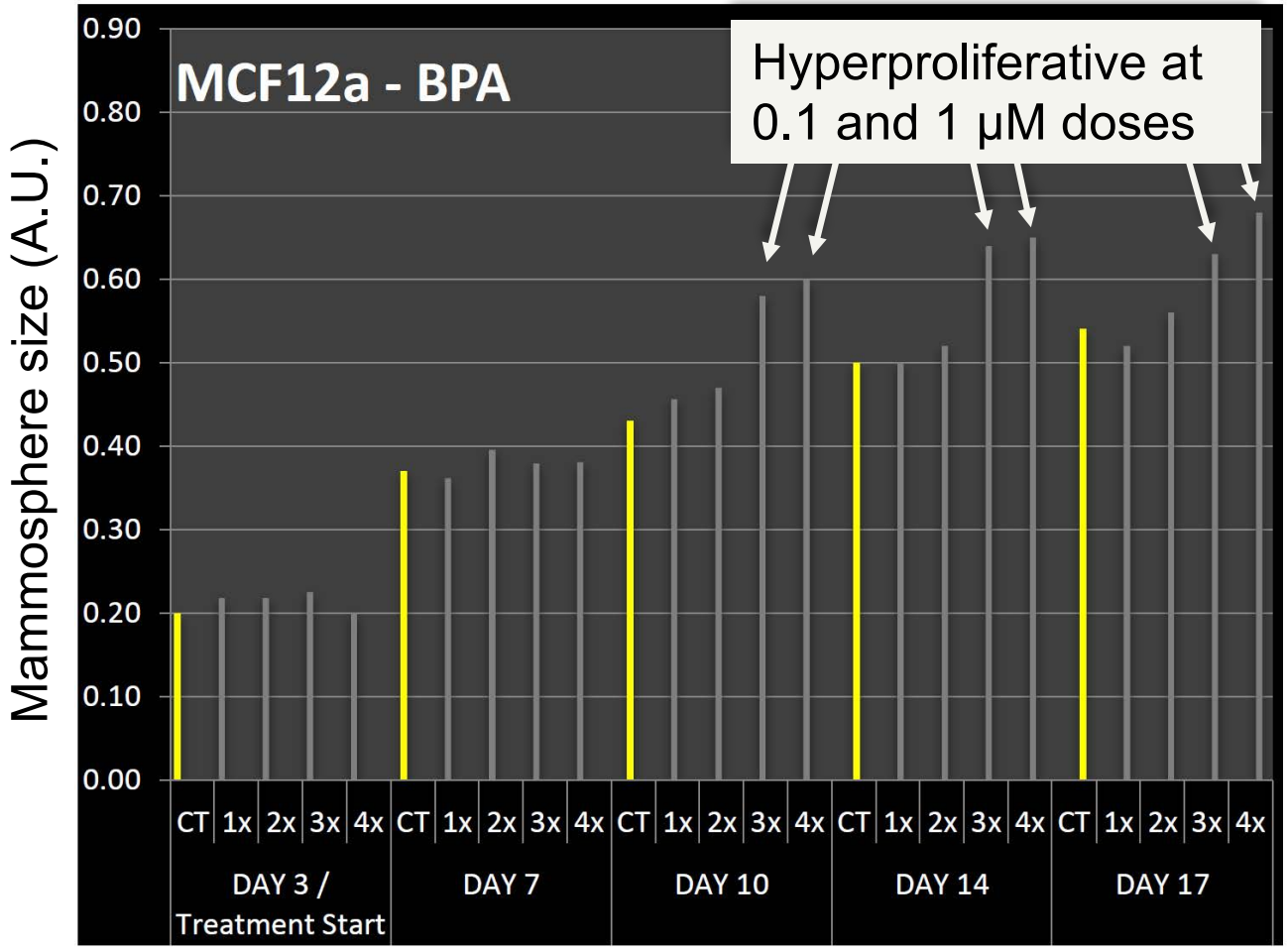




- 96-well footprint
- Magnetically attaches to the stand between imaging sessions
- Holds bioreactor cartridge with 6 culture chambers that are on 9-mm grid
- Pump and valve assemblies are magnetically attached to the plate
- Compatible with conventional microscopy and ImageXpress Micro XLS High Content Analysis system

Markov et al, *Lab on a Chip*, 2012
Markov et al., *Biomedical Microdevices*, 2014

MCF-12A CELLS IN THE BIOREACTOR EXPOSED TO INCREASING DOSES OF BPA



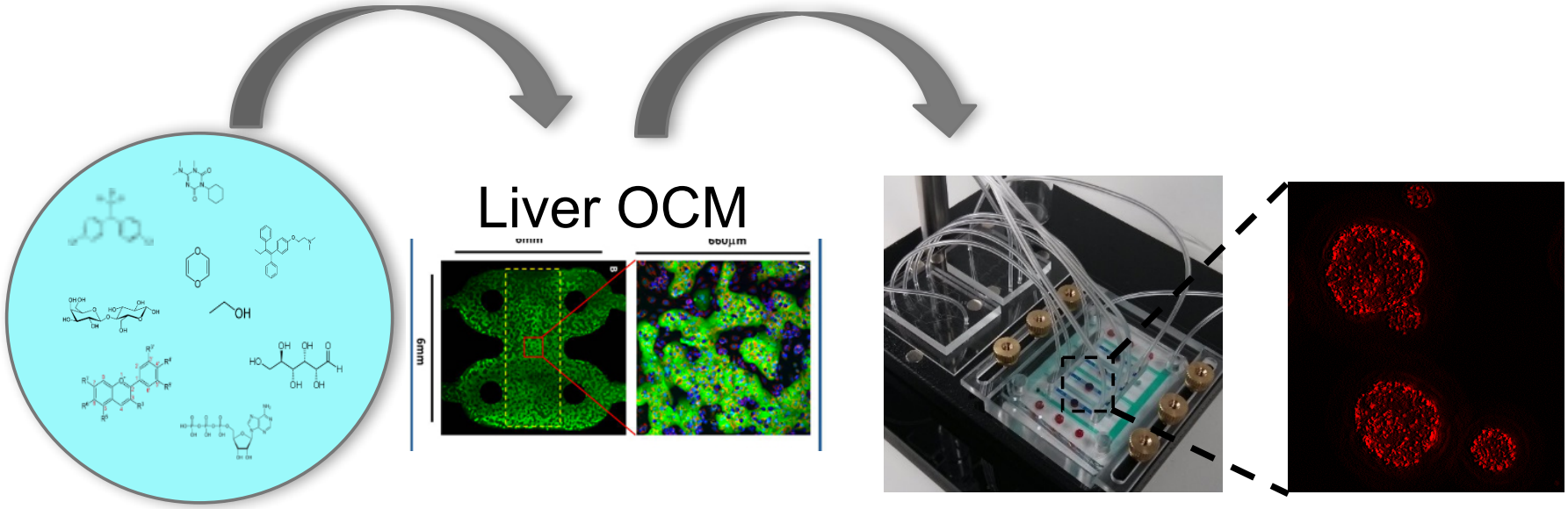
1x, 2x, 3x, 4x = 0.001 μM, 0.01 μM, 0.1 μM, 1 μM

Note: These cells are not as responsive to low doses of BPA (0.01 μM); however, they retain a more typical expression of hormone receptors

10/28/19

ORGAN TO ORGAN INTEGRATION: LIVER-MAMMARY COUPLING

Does Liver Metabolism Effect Toxicity of Compound?



MEDIA COMPATIBILITY: MAMMARY OCM DOWN STREAM OF LIVER

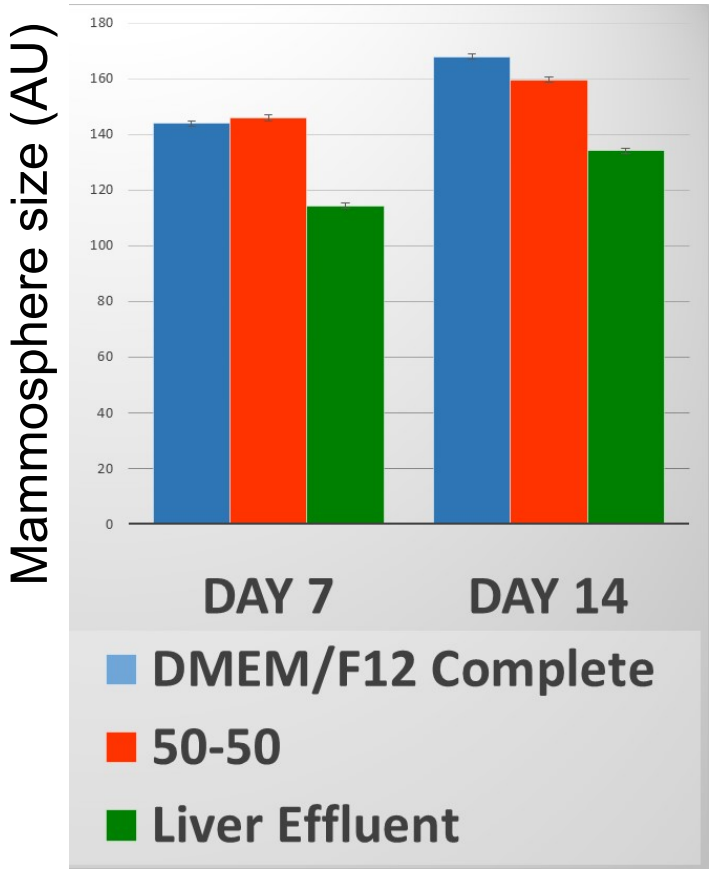
1) Is Liver-OCM compatible with mammary cell medias? (Yes!):

2) Do MG cells retain morphogenic program when exposed to media conditioned by Liver-OCM?

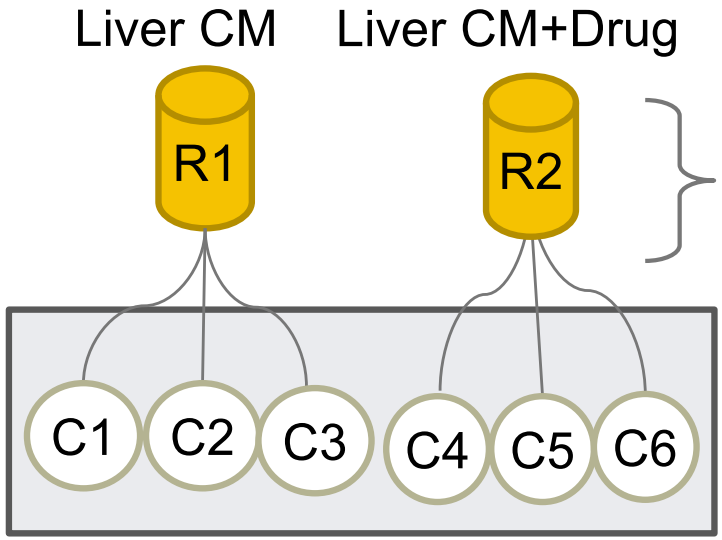
Vanderbilt shipped DMEM/F12 Complete Media to Pittsburgh. ~ 80 mls of Liver-OCM Conditioned DMEM/F12 Complete Media Prepared in Pittsburgh, aliquoted, frozen and shipped back to Vanderbilt for testing

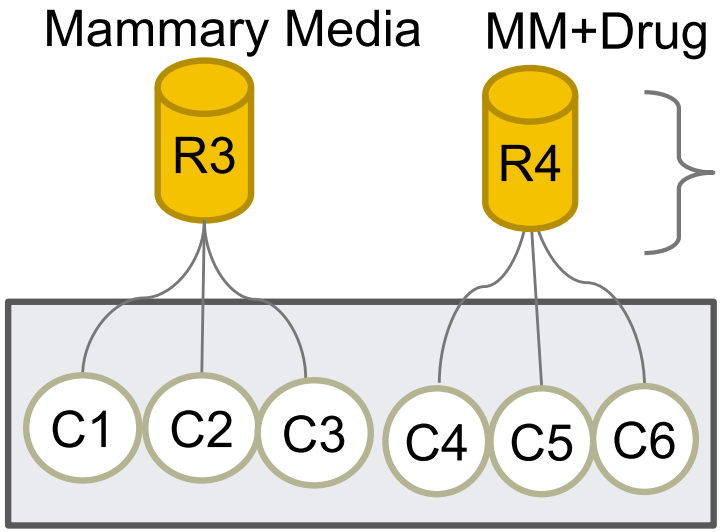
Short term testing on cell line monolayers (4 days) - maintained cell viability.

Long term testing on mammosphere formation demonstrated that a 50% Naïve Media/ 50% Liver Effluent mix maintained proper mammosphere formation and growth



FUNCTIONAL COUPLING: CONFIGURATION FOR TESTING LIVER-CONDITIONED MEDIA + DRUG (CM+D) IN MAMMARY GLAND OCM





Chip1: Liver CM or Liver CM+D are "pre-mixed" with fresh media 50/50.

Media type	UPDDI Liver CM	Vanderbilt Fresh media	Total volume for ~20 days
R1 Liver CM	7.5 ml	7.5 ml	15 ml
R2 Liver CM + Drug	7.5 ml (2X drug conc.)	7.5 ml	15 ml

Chip 2: Control with Fresh Media ± Drug.

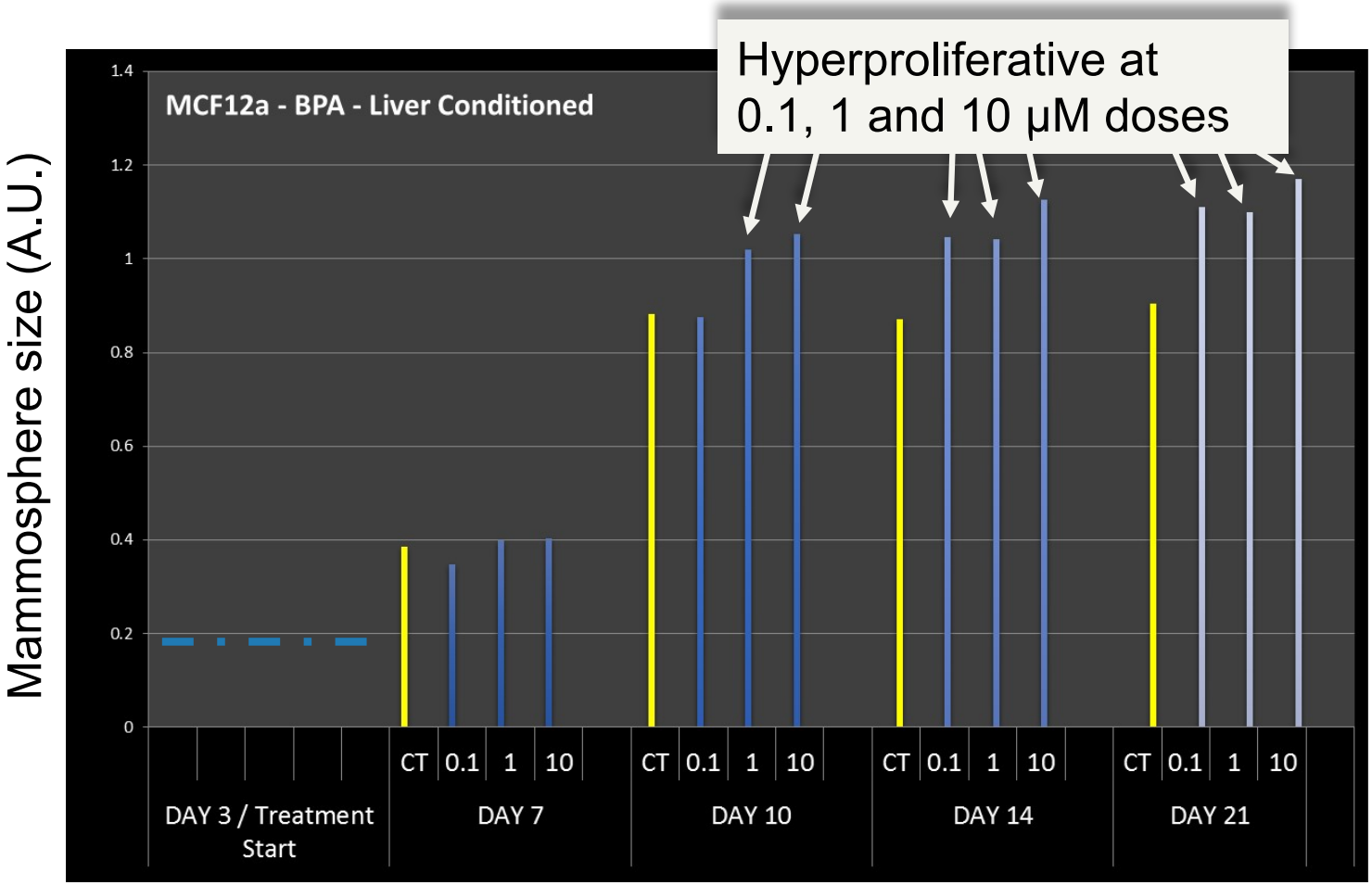
Media type	UPDDI Liver CM	Vanderbilt Fresh media	Total volume for ~20 days
R3 Liver CM	-	15 ml	15 ml
R4 Liver CM + Drug	-	15 ml (1X drug conc.)	15 ml

Vernetti, Markov, Fryman, Bazilevich

COMPOUNDS FOR TESTING FUNCTIONAL AND PHYSICALLY COUPLING OF LIVER & MAMMARY OCMS

Compound	cLogP	Liver Metabolism	Cmax
Genistein	3.04	High clearance compound, hydroxylation metabolites, glucuronide conjugates	Cmax = 1.8 µg/ml (6.7 µM)
Bisphenol A	3.43	High clearance compound, glucuronide conjugate	Monkey Cmax = 107 ng/mL (0.49 µM)
DES	4.62	Excreted in urine and feces, principally as the glucuronide with biliary excretion. Enterohepatic recirculation of DES after bacterial hydrolysis in the distal colon results in the prolonged plasma levels.	Human Cmax = 3.4 ± 1.93 ng/mL (12.6 nM)

MCF-12A CULTURED WITHIN BIOREACTOR WITH CONDITIONED LIVER MEDIA +/- INCREASING BPA



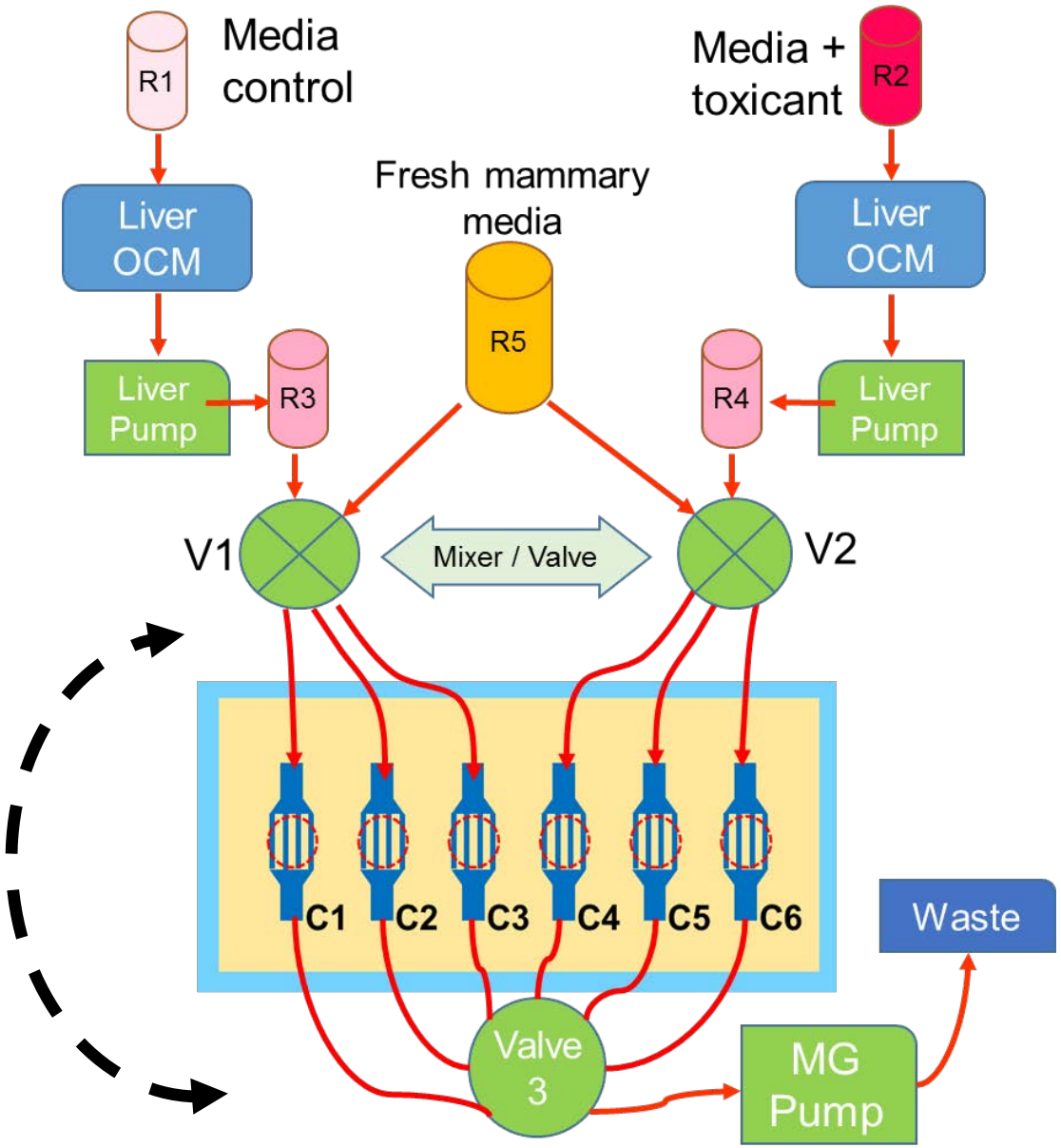
Liver was exposed to 0.2, 2 and 20 µM BPA. CM collected and shipped to Vandy where it was mixed 50:50 with fresh naïve media to yield 0.1, 1 and 10 µM for MG-OCM.

Vernetti, Markov, Fryman, Bazilevich **10/28/19**

OCM Integration: UPitt Provided Liver OCM Conditioned Media for Functional Coupling Experiments

Project	Volume Provided	Naïve and Treated Media Provided
Project 1 (Mammary Gland)	12 X 32 ml	Genistein: 0, 0.4, 4, 40 µM BPA: 0, 0.4, 4, 40 µM DES: 0, 0.004, 0.04, 0.4 µM
Project 2 (Chondrogenesis)	4 X 135 ml	Valproic Acid: 0, 100, 1000 and 2000 µM
Project 3 (Fetal Membrane)	8 X 50 ml	TCDD: 0, 6.6 µM
Project 5 (Microclinical Analyzer)	3 X 500 µl	1- naïve media 2- 10 mM acetaminophen 3- 100 uM ascorbic acid 4 - 1.4 mM acetaminophen 5 - 100 µM ascorbic acid 6 - 400 µM uric acid

SCHEMATIC FOR ONGOING DIRECT COUPLING LIVER-OCM TO MG-OCM



One Liver-OCM feeding into 3 cell chambers of mammary chip.

Thus, two Liver Chips to feed 1 Mammary Gland Chip.

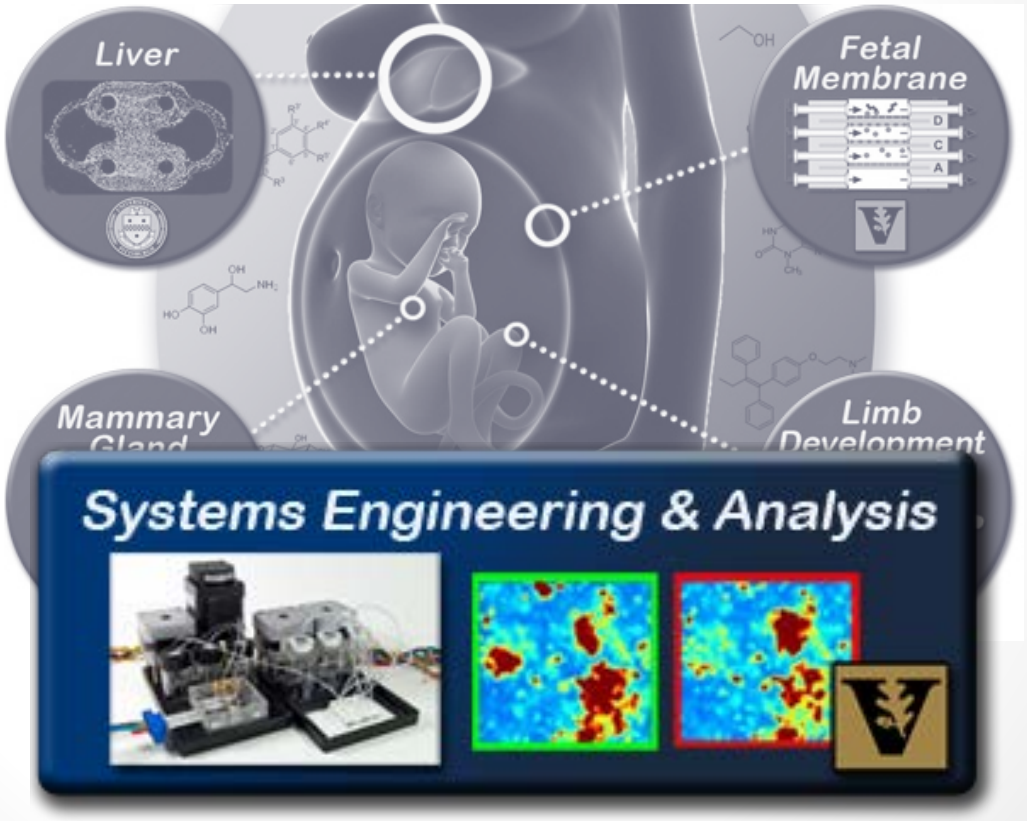
One possible configuration using ± drug conditions where ***Liver effluent is "mixed" 50/50 with fresh Mammary media post Liver-OCM.***

Why? Prior results suggest using Liver CM straight has a slight effect on mammosphere formation and growth.

LESSON #5

One can link OCMs together to allow for hepatic clearance (or metabolic activation) of xenobiotics, but it requires careful consideration of both media compatibility and functional scaling.

PROJECT 5 – SYSTEMS ENGINEERING & ANALYSIS



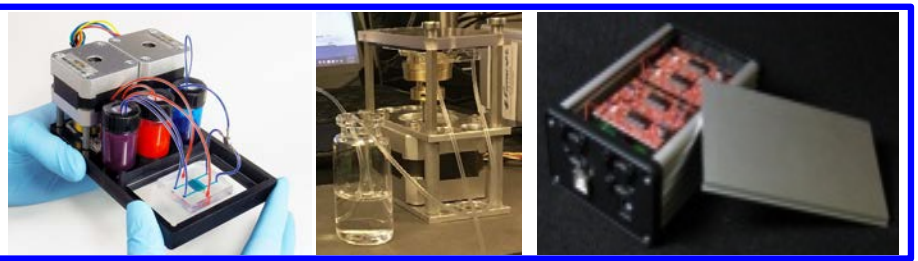
John Wikswo, Co-PI
John McLean
David Cliffel
Simona Codreanu
Stacey Sherrod
Greg Gerken
Clayton Britt
Shane Hutson
Alex Auner
Kazi Tasneem

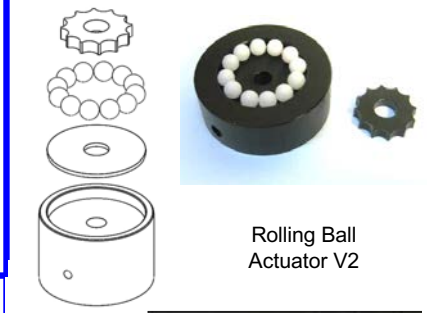
10/28/19

5.1: FASTER, BETTER, CHEAPER

THE NEXT GENERATION OF VIIBRE NVU HARDWARE IS COMING ONLINE: PUMPS

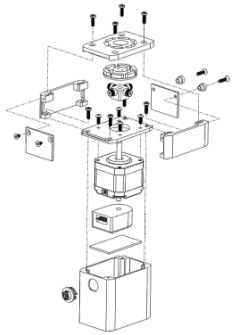
- V2.0
  - Open frame
  - Hard to sterilize
  - External controller with cables

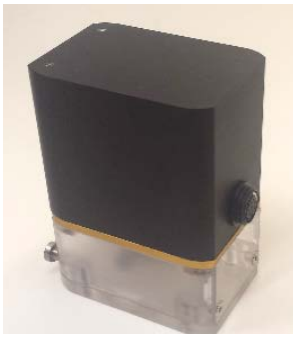


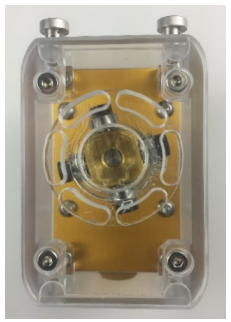


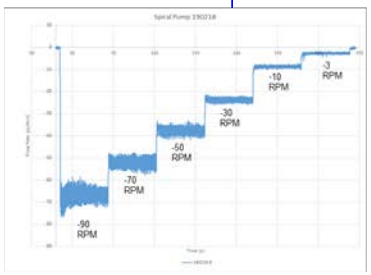
Rolling Ball Actuator V2

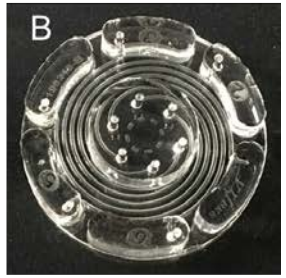
- V3.0
  - Totally enclosed
  - Wipe-sterilizable
  - Through-plate fluidics
  - External controller





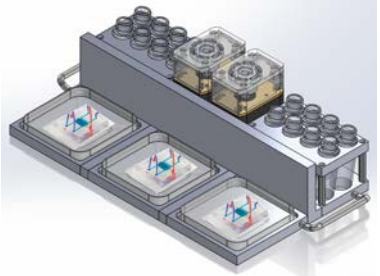




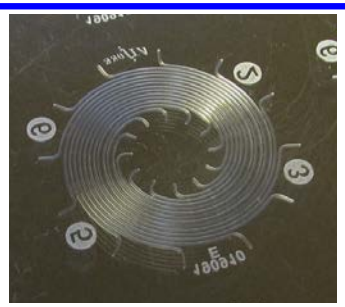


Six-Channel Spiral Pump

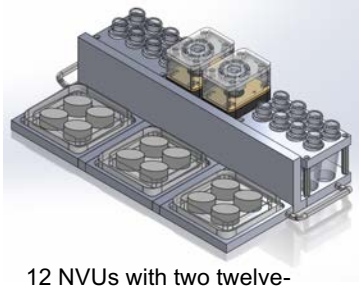
Classic NVU with 2 pumps



3 NVUs with six-channel spiral pump and 4x6 PK valve



Twelve-channel spiral pump



12 NVUs with two twelve-Channel Spiral Pumps

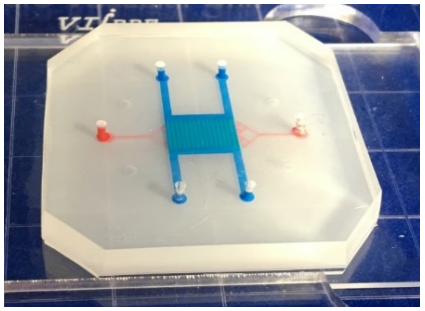
1) V3.5 wireless controller.
2) Twelve-channel pumps
3) Puck NVUs
4) Increased throughput

NVU is used by one of the newly funded NAM centers, David Cliffel, PI.

10/28/19

5.1: FASTER, BETTER, CHEAPER

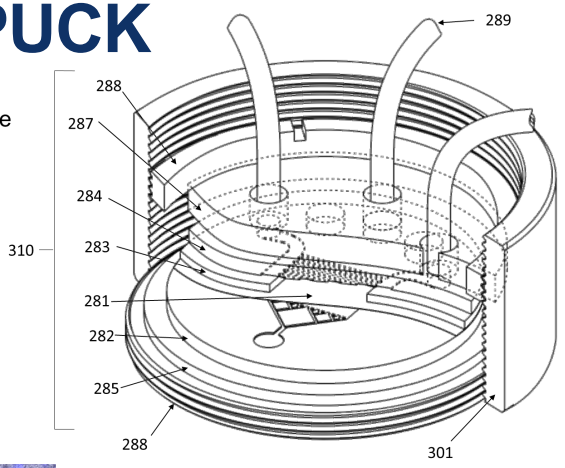
THE NEXT GENERATION OF VIIBRE NVU HARDWARE IS COMING ONLINE: PUCK

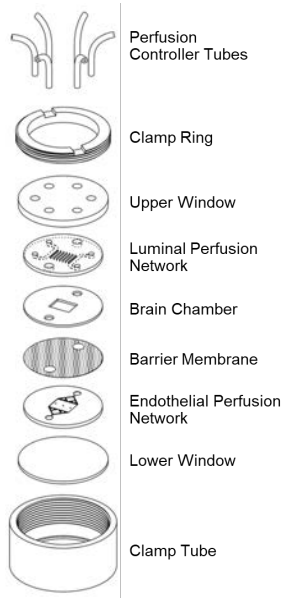


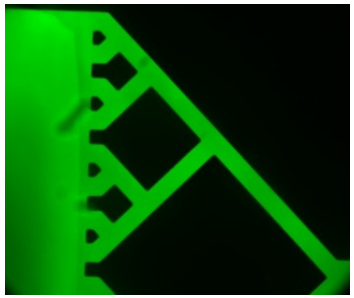
Classic NVU

Puck NVU

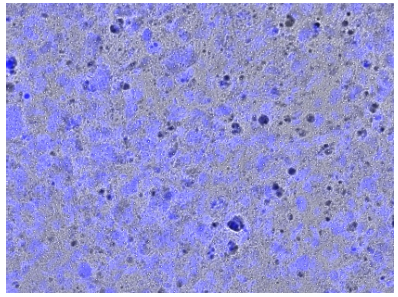
- Key features
  - 301 – Thorlabs lens tube
  - 288 – retaining ring
  - 287 – transparent pressure plate
  - 283 – neural cell chamber
  - 281 – membrane
  - 282 – endothelial chamber
  - 285 – glass bottom



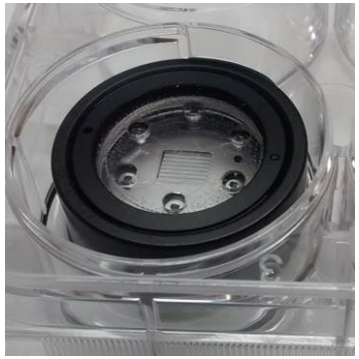




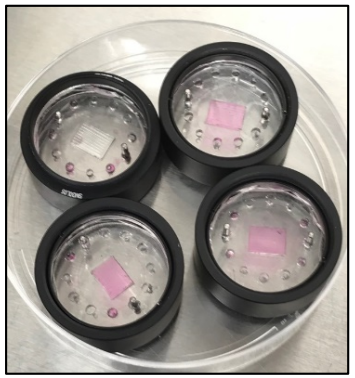
NVU vascular-side perfusion splitter



Astrocytes after disassembly



NVU Puck in a six-well plate



Gut Pucks loaded open-faced and sealed

Access to individual cellular compartments makes device amenable to multi-omic sample analysis
Cell numbers renders device compatible with multiomic analysis: Protein yield of ~5-10 µg per Puck
Assemble wet after layers confluent
Monitor effluent kinetically
Dissect intracellular molecular events
Disassemble before fixing

Puck development funded by DTRA, NIH/NCATS and DARPA

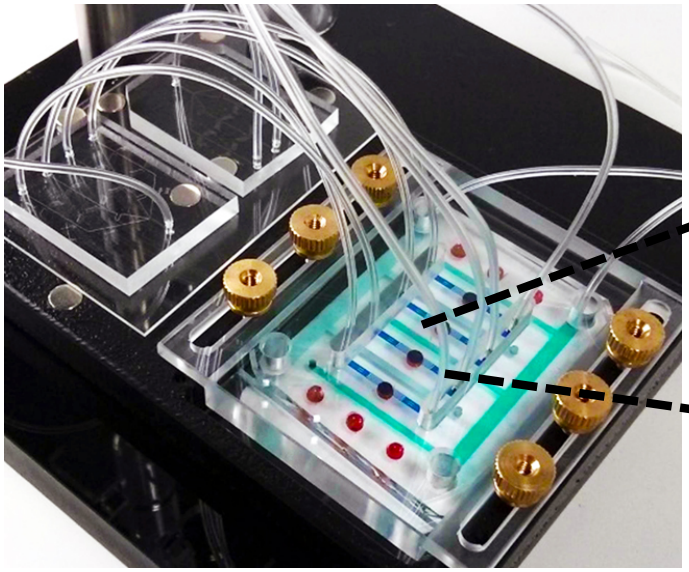
LESSON #6

Get a good “plumber”!

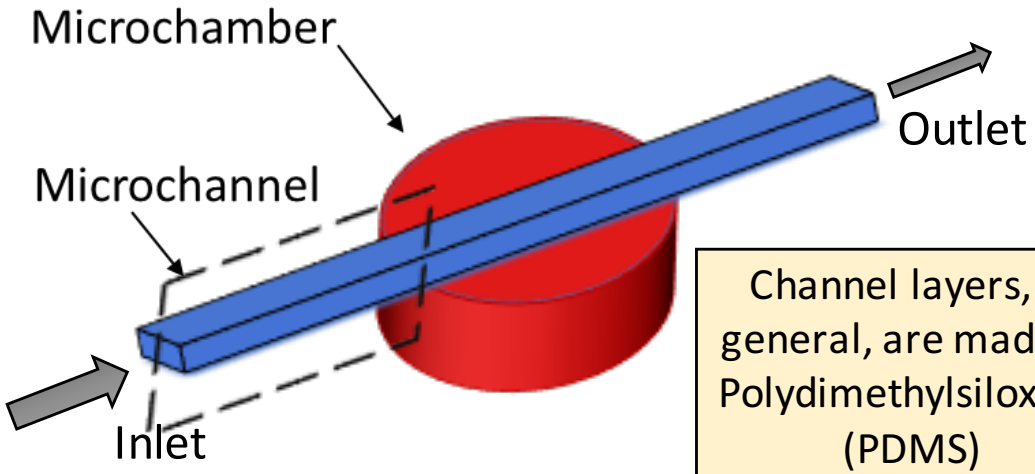
The behind-the-scenes engineering needed to make OCMs work effectively, reproducibly and at a reasonable cost is immense.

5.2: IN-DEVICE TOXICOKINETICS

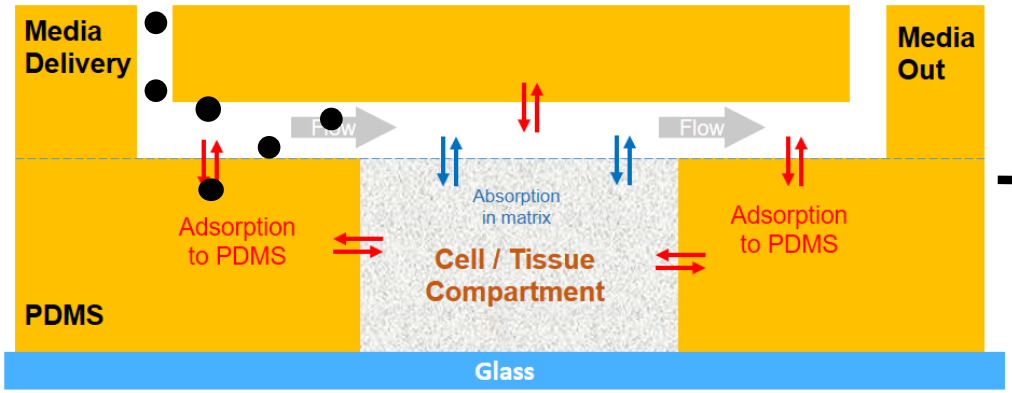
Hydrophobic chemicals can partition into PDMS.



Markov et al., 2012



Channel layers, in general, are made of Polydimethylsiloxane (PDMS)

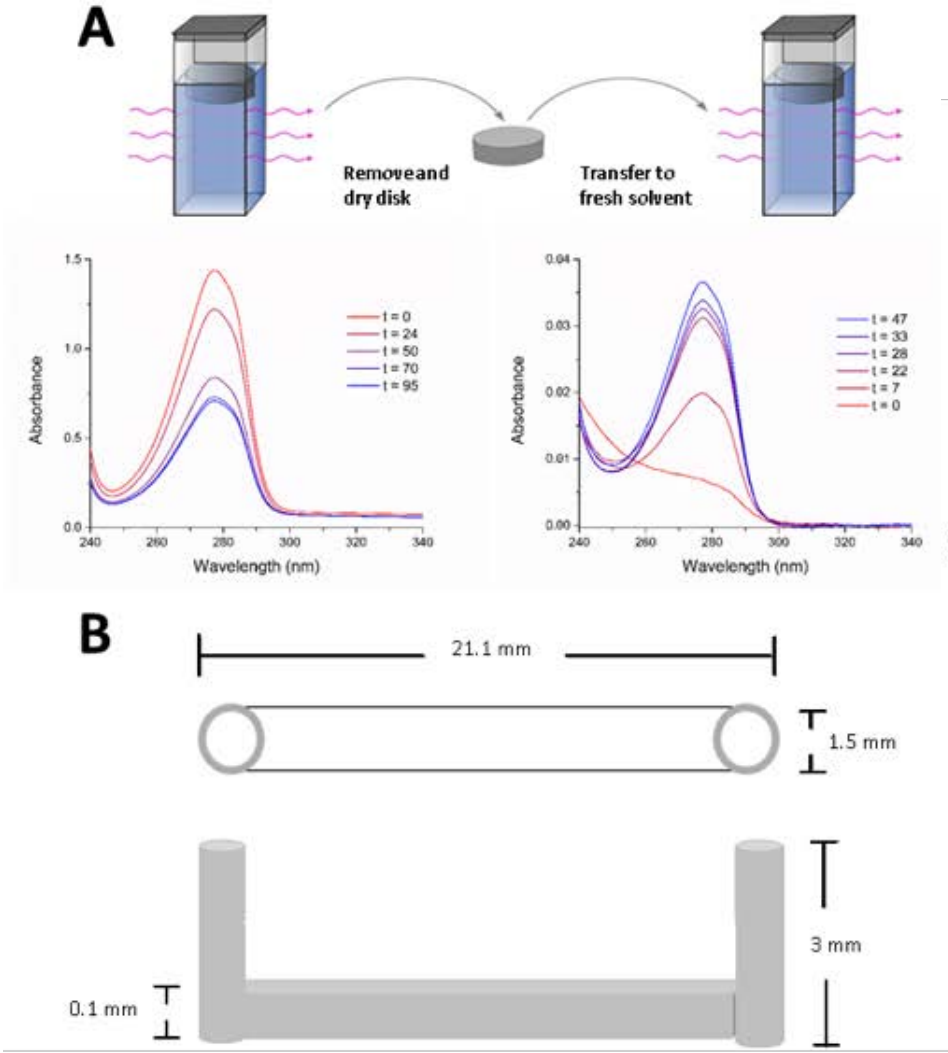


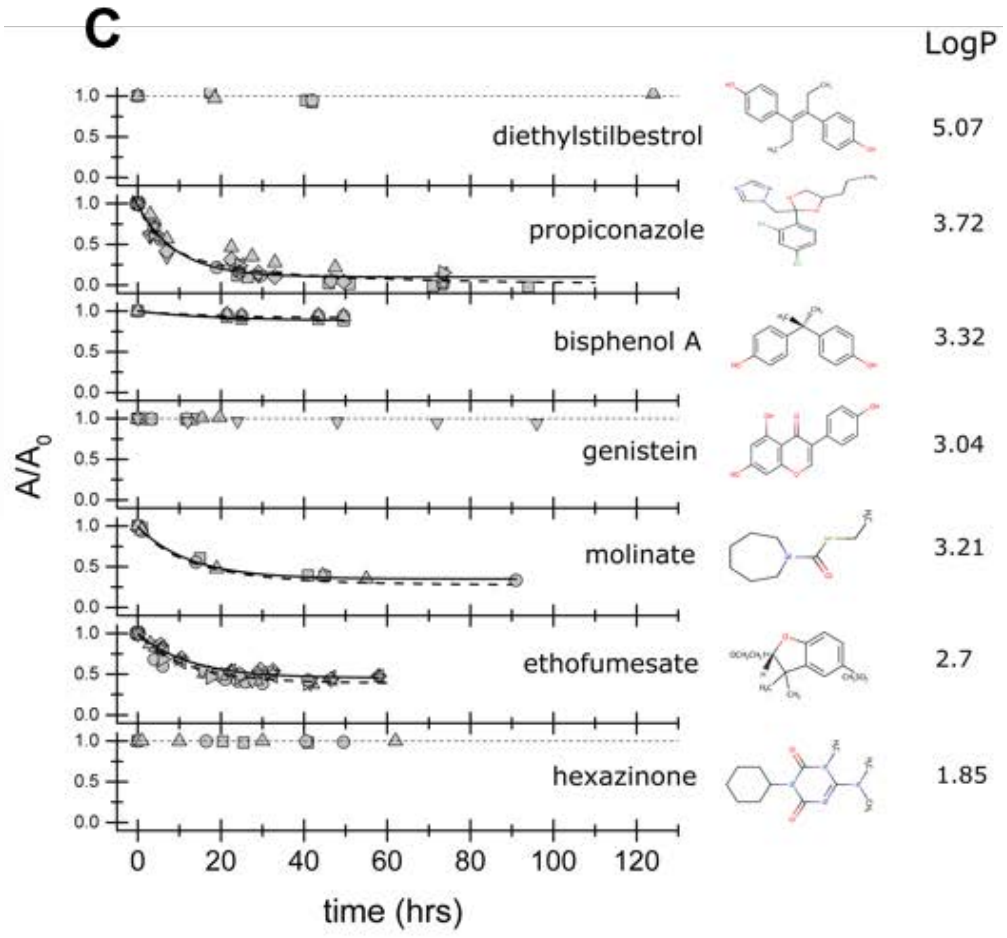
D.A. Markov, L.J. McCawley

- What are the **actual concentrations** to which cells are exposed?
- What are the **saturation dynamics** of PDMS surface?
- What are the effects of different **dosing schemes**?

5.2: IN-DEVICE TOXICOKINETICS

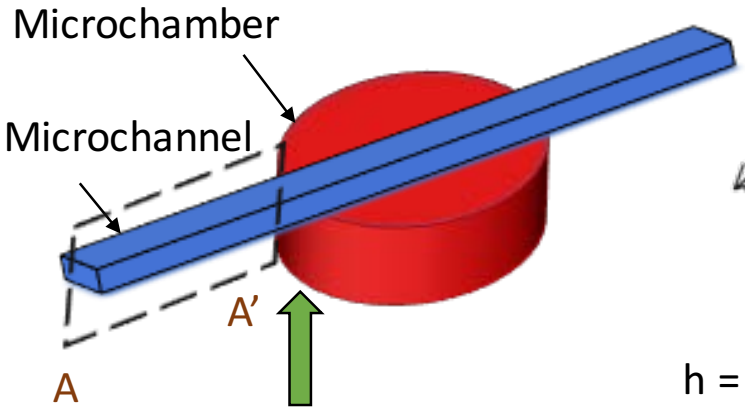
Measuring chemical-PDMS interaction kinetics

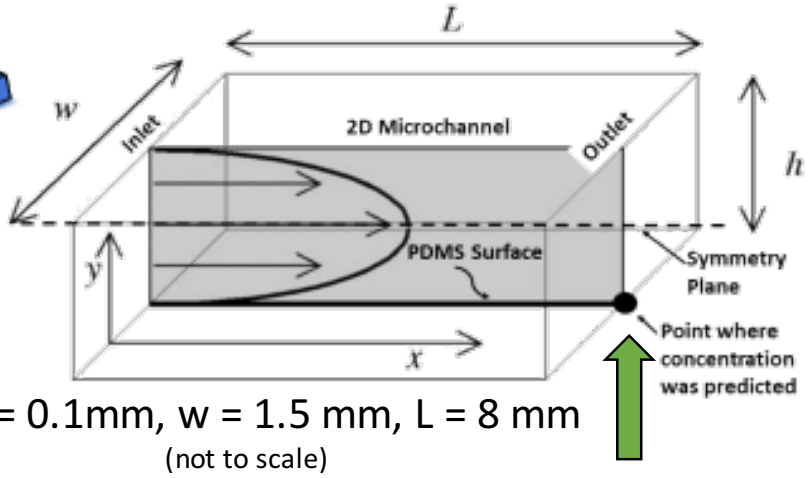




5.2: IN-DEVICE TOXICOKINETICS

Modeling chemical-PDMS interactions



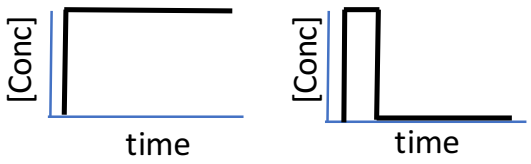


h = 0.1mm, w = 1.5 mm, L = 8 mm
(not to scale)

Model run for tested chemicals:

- **Ethofumesate**: reversible binding with PDMS surface
- **Propiconazole**: irreversible binding with PDMS surface
- **Rhodamine B**: lesser extent binding with PDMS surface

Dosing Schemes



Continuous exposure

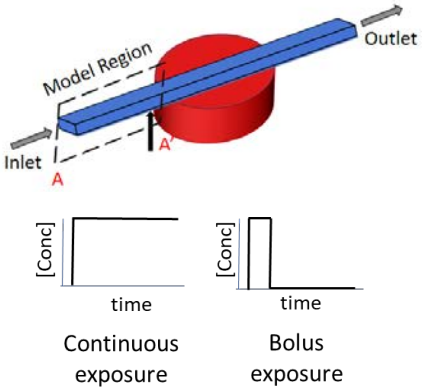
Bolus exposure

Model output

- Chemical exposure was taken as concentration above the PDMS surface at the end of microchannel
- Fraction of surface saturation

5.2: IN-DEVICE TOXICOKINETICS

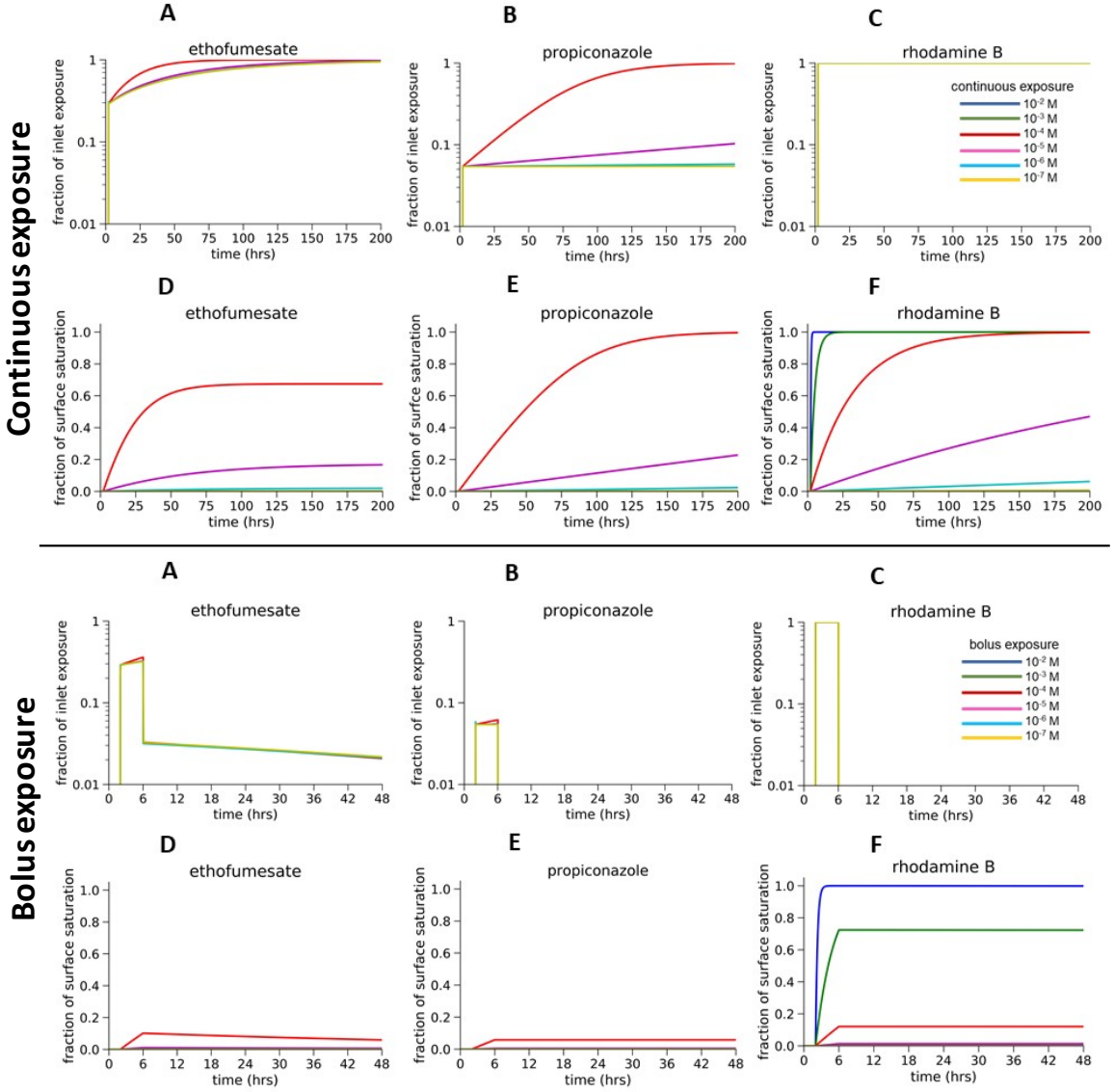
Modeling chemical-PDMS interactions



Ethofumesate:
reversible binding with PDMS surface
Propiconazole:
strong affinity to PDMS surface
Rhodamine B:
Small extent binding by PDMS surface

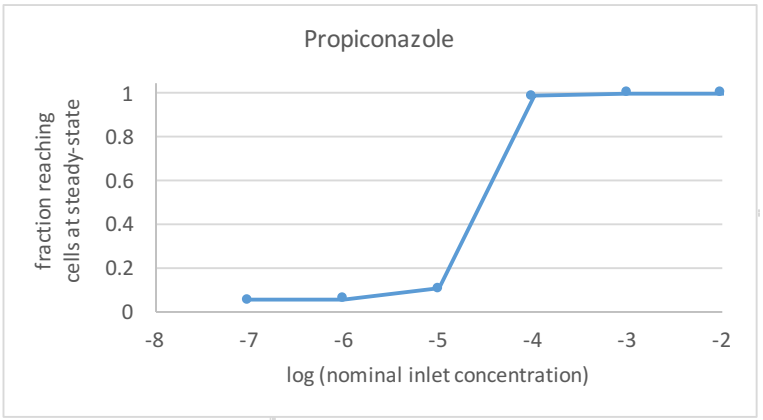
Model Key Outcomes:

- For chemicals that reversibly bind, a bolus dose at the inlet may translate into an extended exposure for cells in the device due to delayed release of the chemical from PDMS surfaces.
- For chemicals with strong affinity to PDMS, the actual exposure may be an order of magnitude less than the nominal inlet concentration.

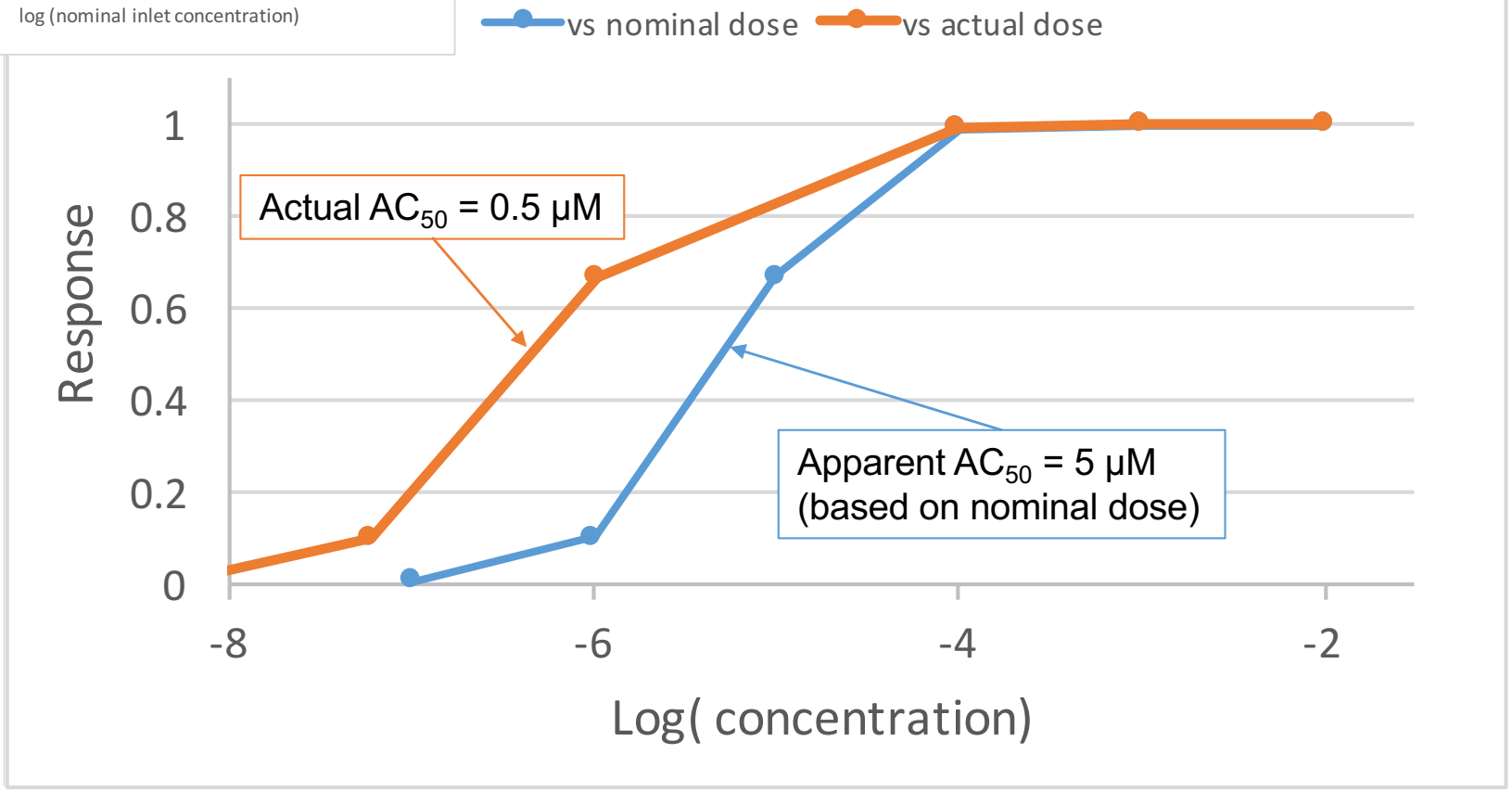


Auner et al, *Lab on a Chip* 2019

5.2: IN-DEVICE TOXICOKINETICS



Need in-device toxicokinetics to get the correct dose-response curves



LESSON #7

If OCMs are among the next-generation NAMs, the community will need measurements and models for in-device toxicokinetics.

You have to know the *in vitro* dose-response curve before you can extrapolate to predict *in vivo* effects.

BONUS LESSON: GRATEFULLY ACKNOWLEDGE THE PEOPLE THAT MAKE ALL THIS HAPPEN!

10/28/19