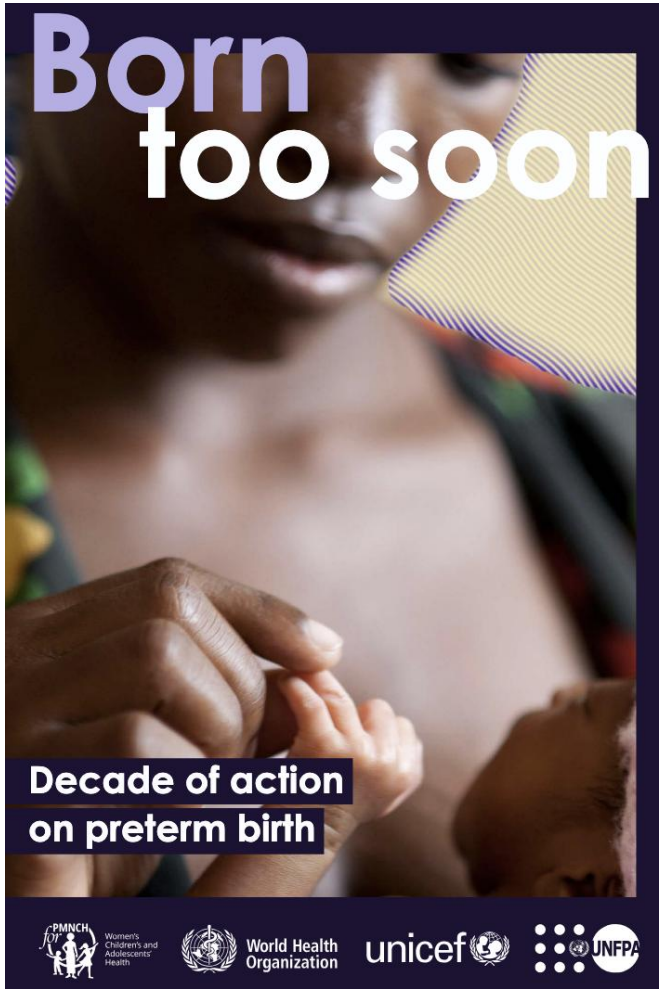
Organ-on-Chip Models of the Feto-Maternal Interface: Enabling Rapid Hazard Analyses of Environmental Contaminants Impacting Pregnancy

Prof. Arum Han, Texas A&M University

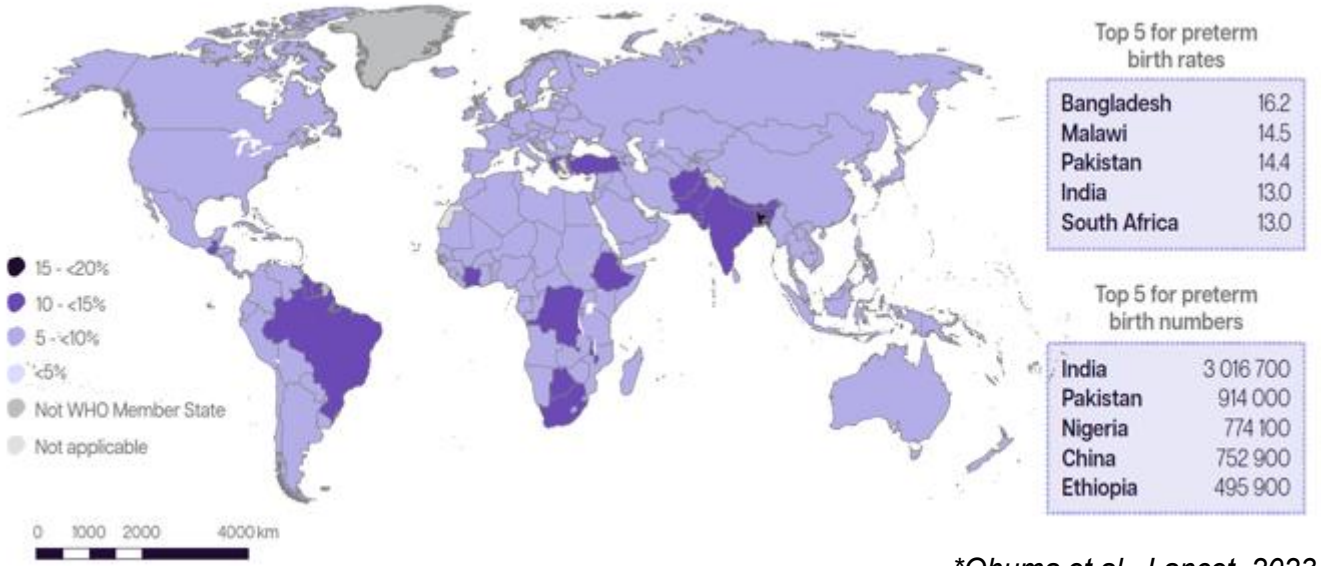
NIEHS Superfund Research Program (SRP) Risk e-Learning Webinar Series
From Cells to Solutions: Emerging Tools for Studying Health and Disease
Session II 3D Models and Technologies to Illuminate Biological Effects of Contaminants

Preterm Birth (PTB) and Adverse Pregnancy Outcomes (APOs)



Global PTB rate

150 million babies were born prematurely in the last decade (~11% of all pregnancies end preterm worldwide)

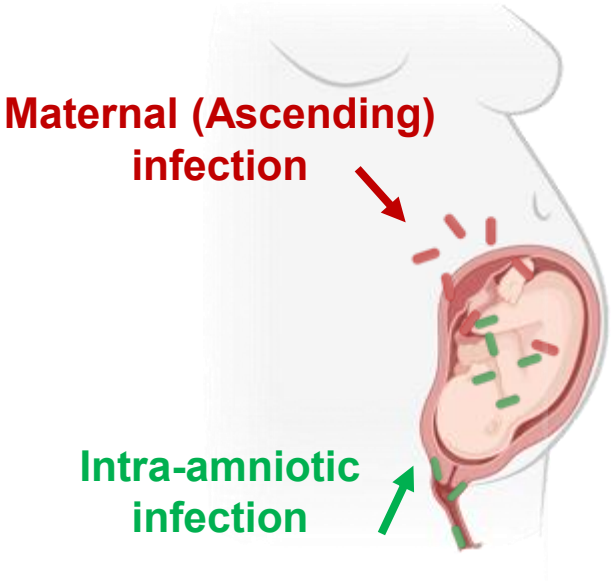


**Ohuma et al., Lancet, 2023*

- **Need to understand the mechanism**
- **Identify high-risk pregnant subjects**
- **Provide proper intervention**

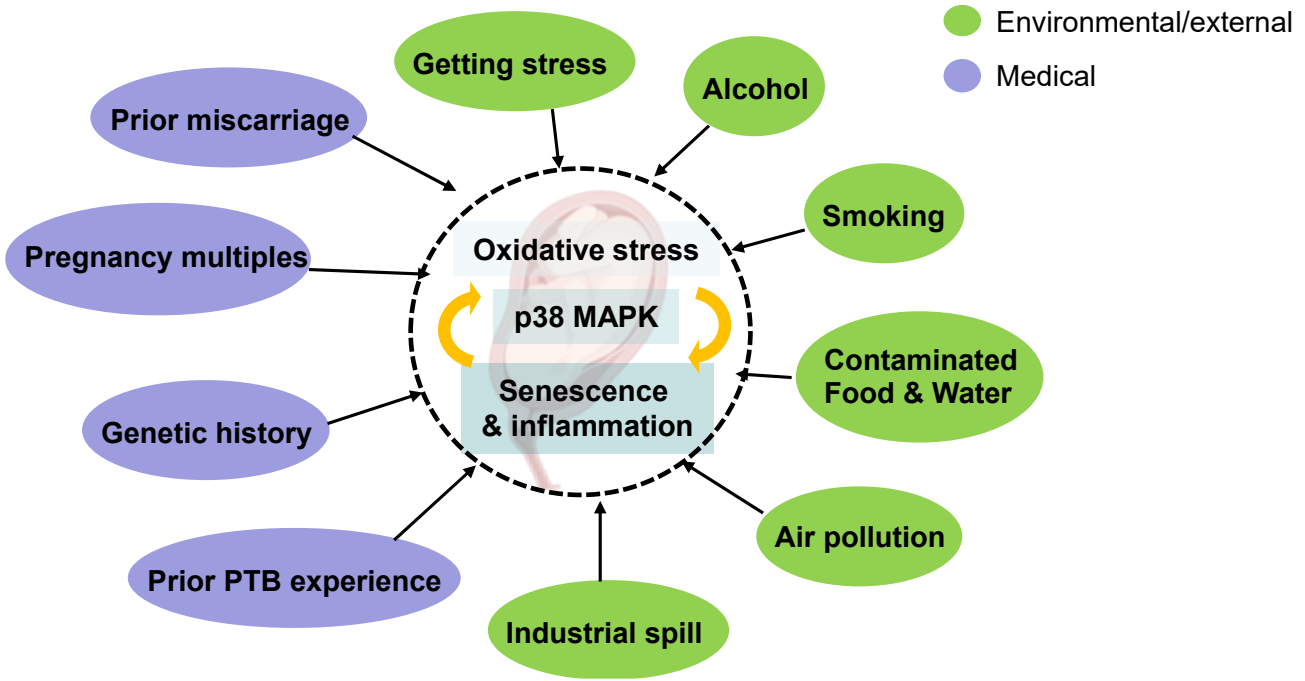
Preterm Birth (PTB): Complex Etiology

Infection-associated Inflammation



- Infectious-associated inflammation cause **40%** of preterm birth
- Multiple environmental/medical risk factors for sterile inflammation

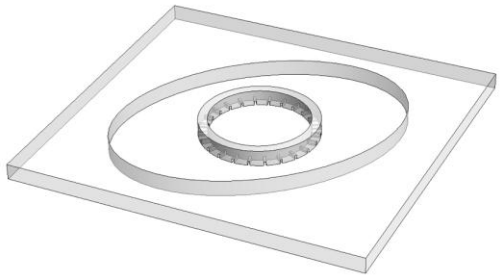
Risk factors inducing Non-infectious (sterile) inflammation



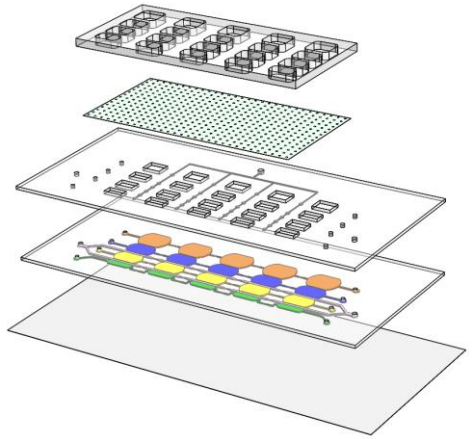
➢ **Comprises feto-maternal interfaces**

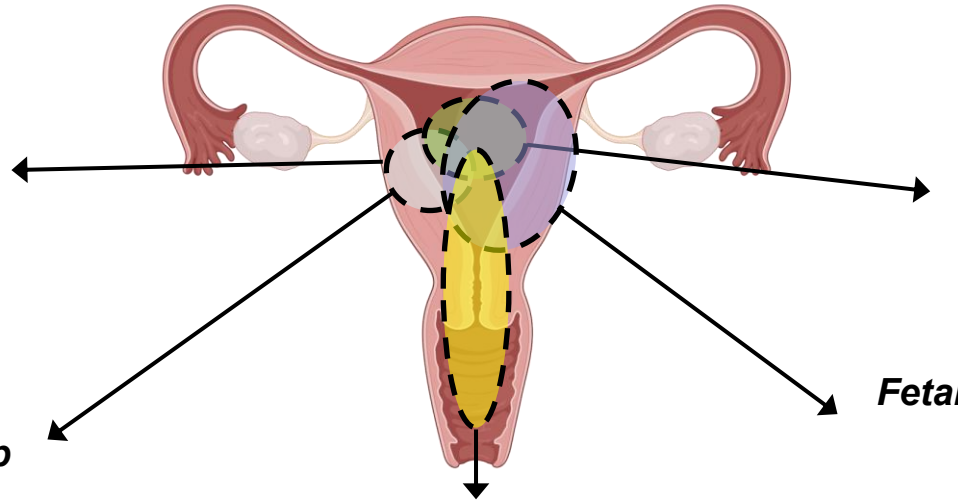
Our MPS Models of Female Reproductive Tract

Amnion membrane Organ-on-Chip (AM-OOC)

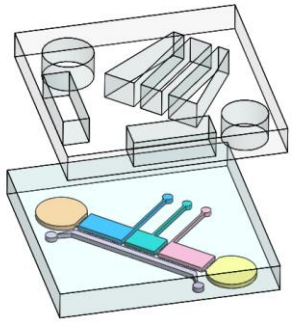


Arrayed fetal membrane Organ-on-Chip (Arrayed FMi-OOC)

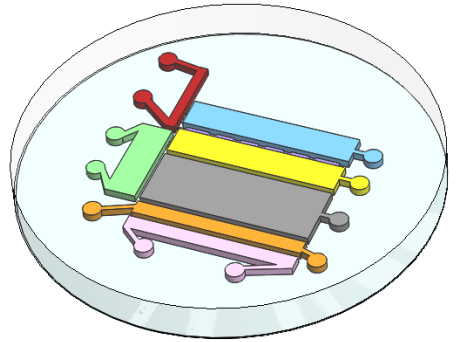




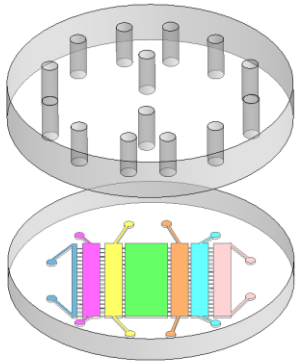
Vagina-cervix-decidua Organ-on-Chip (VCD-OOC)



2nd trimester Placenta Organ-on-Chip (PLA-OOC)



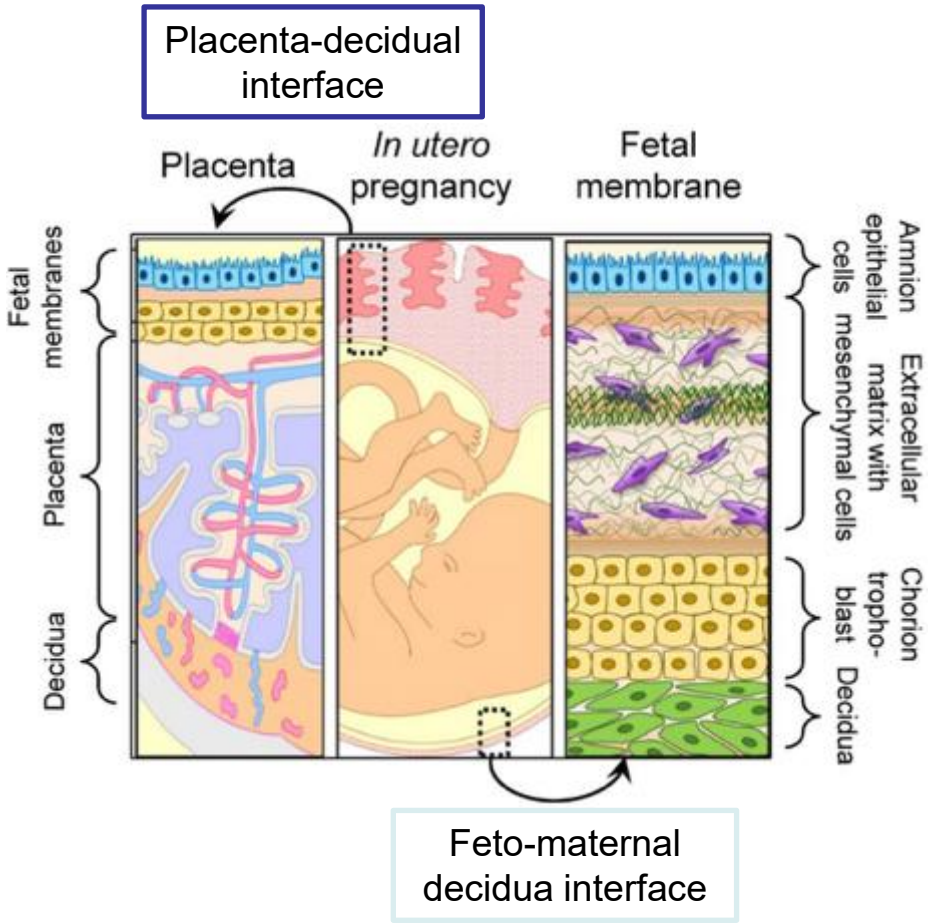
Fetal membrane-Placenta-Organ-on-Chip (FMi-PLA-OOC)



Ramkumar Menon, Ph.D.
Professor of Obstetrics & Gynecology
University of Texas Medical Branch at Galveston

MPS Models for Pregnancy and Women's Health

Complexity of feto-maternal interface



Challenges of current models to study feto-maternal interface

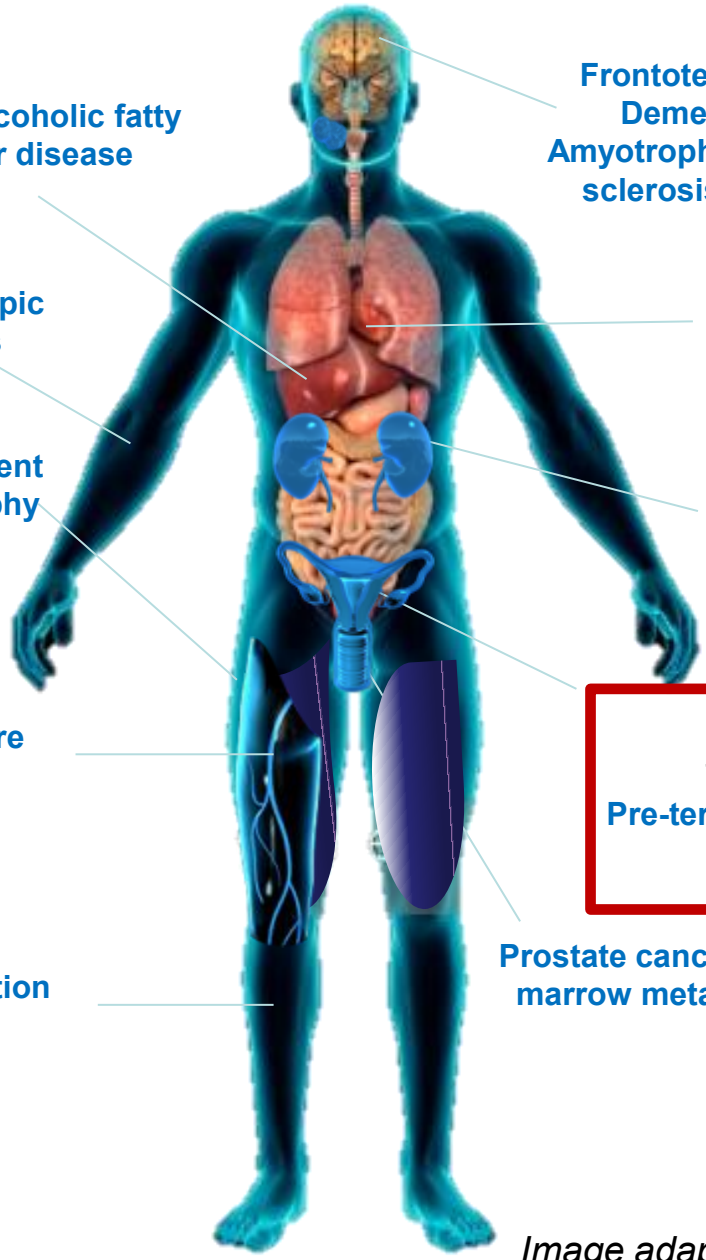
- ***2D and explant human cell cultures***, typically grown in trans-wells, neither embody the intrauterine environment, nor maintain the inter-cellular interactions seen *in utero*.
- ***Animal models***, for example a mouse model of FMi, do not structurally mimic human FMi. Specifically, the chorionic trophoblast, which is a barrier from maternal immune cell infiltration to the fetus, is rudimentary in rodents.
- ***Non-human primates (NHPs)*** mimic human pregnancy the best; however, testing drug efficacy and safety is cost prohibitive.
- ***Explant perfusion studies in "at term placentas"*** are restricted to the placenta-decidua interface, and the fetal membrane-decidua interface of FMi is *not* tested. These tissues are partially necrotic, which limits their utility. Also, perfusion models do not replicate disease states, and drug efficacy testing in them is impossible.

NICHD R01 & NCATS ”Clinical Trial on a Chip” (2020-2026)

NICHD R01

Intercellular Interactions define Cell Migrations and Transitions that Maintain Fetal Membrane Homeostasis

(MPI: Ramkumar Menon & Arum Han)



Non-alcoholic fatty liver disease

Frontotemporal Dementia; Amyotrophic lateral sclerosis (ALS)

Pediatric Atopic Dermatitis

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

Dystrophin-Deficient Muscular Dystrophy

Polycystic Kidney disease

Progeria - Premature Vascular Aging

Arum Han & Ramkumar Menon
Texas Engineering Experiment Station
Pre-term birth - Maternal-fetal interface on a chip
NCATS/NICHD joint funding

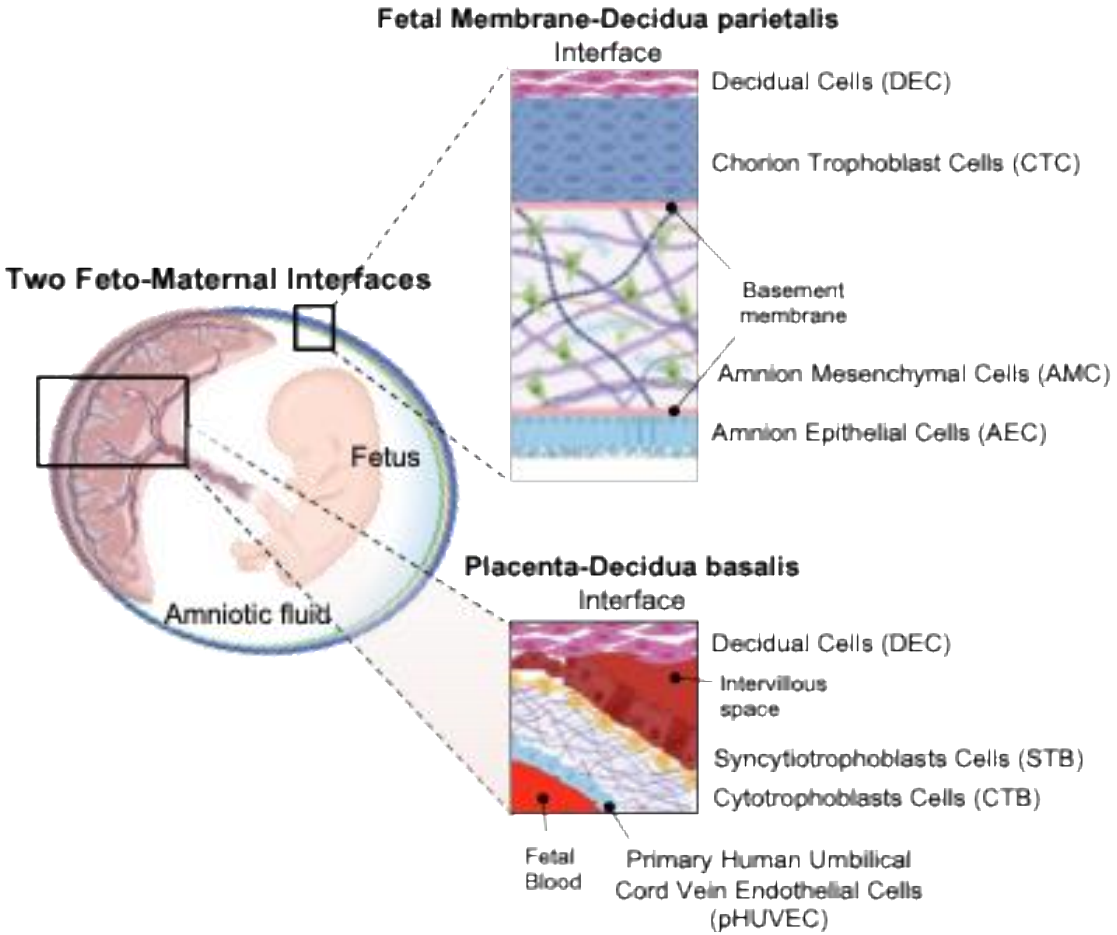
Prostate cancer bone marrow metastasis

Tendon inflammation and fibrosis

Image adapted from NCATS

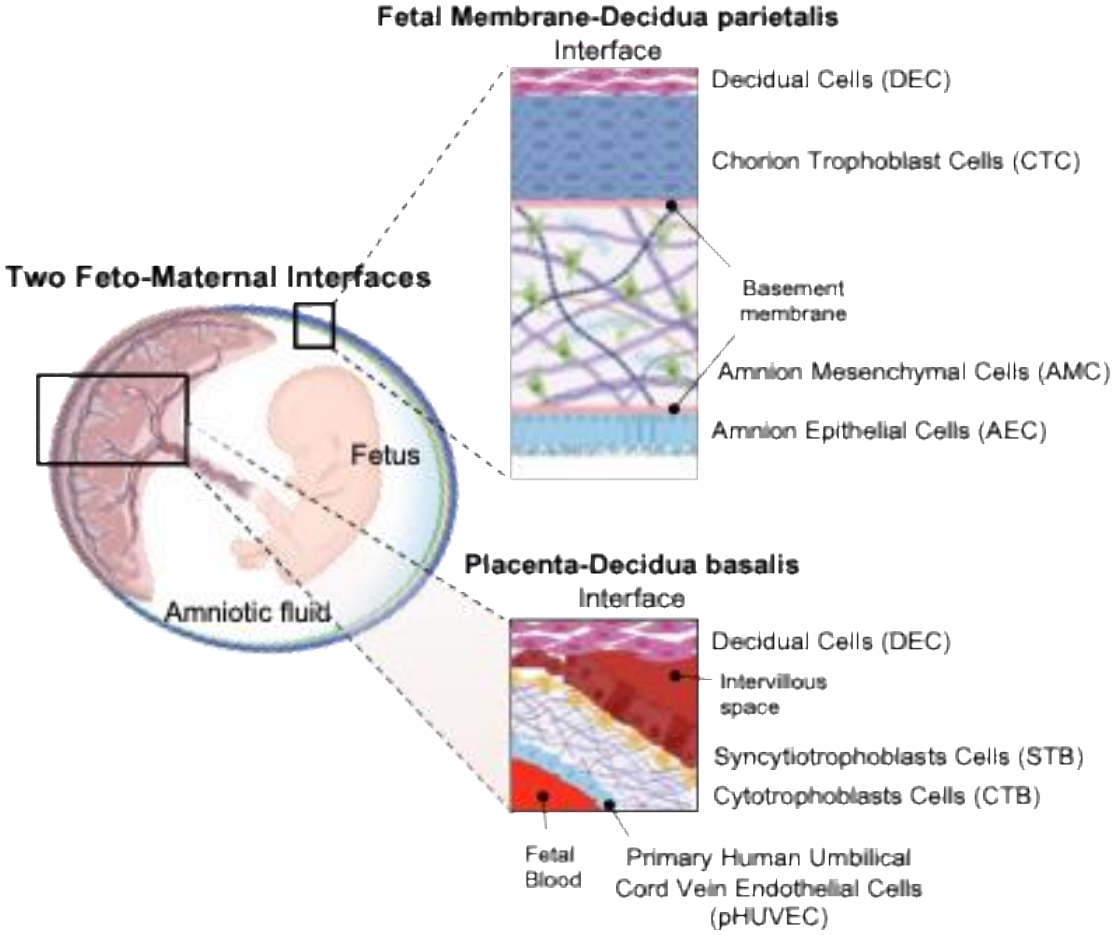
Feto-Maternal Interface Organ-on-Chips

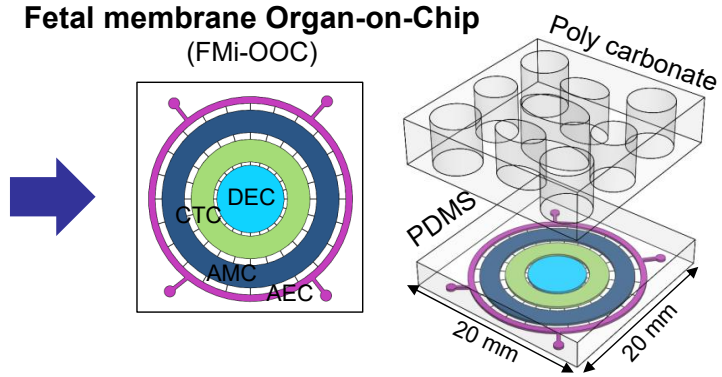
Two Feto-Maternal Interfaces: Fetal Membrane and Placenta Interfaces

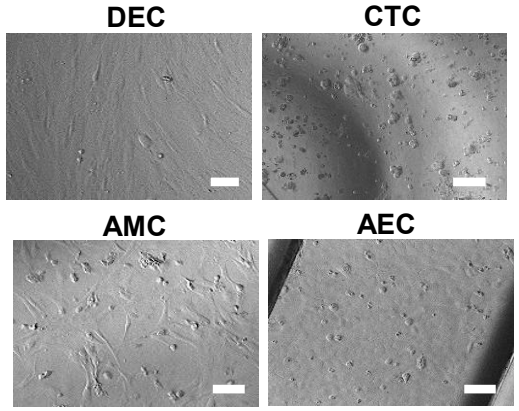


Feto-Maternal Interface Organ-on-Chips

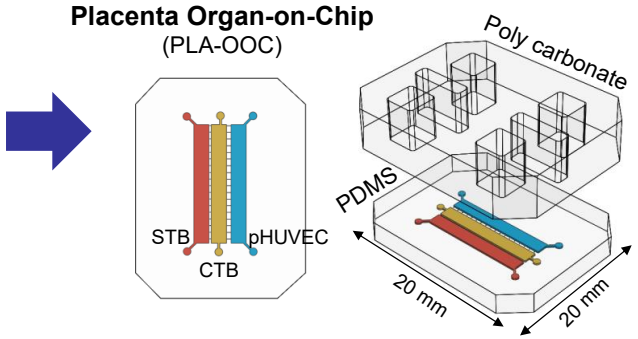
Two Feto-Maternal Interfaces: Fetal Membrane (FMi-OOC) and Placenta (PLA-OOC) Interfaces

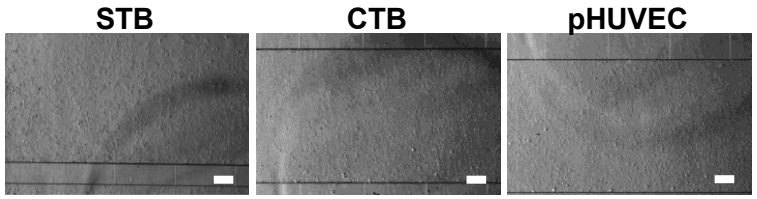


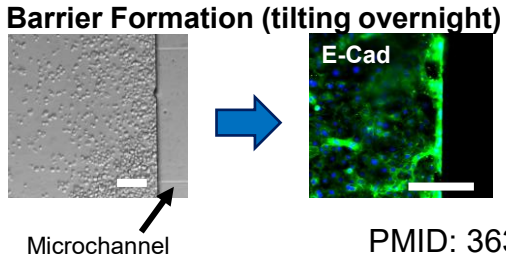




PMID: 33112317



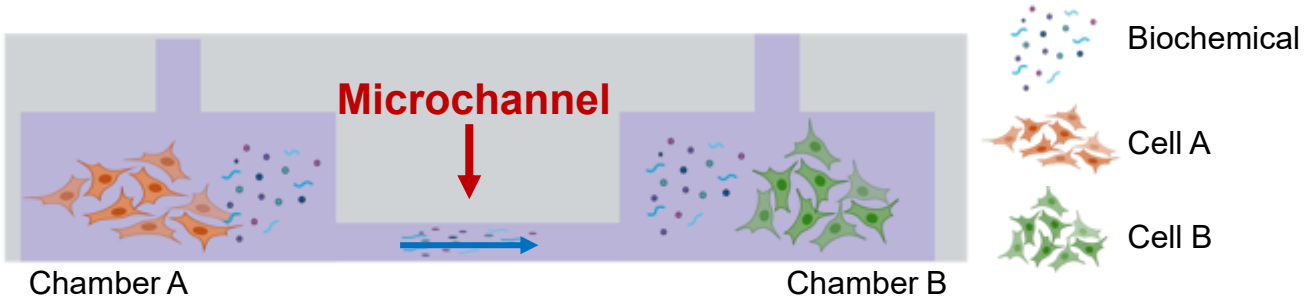




PMID: 36322152

Our MPS Model Key features (1)

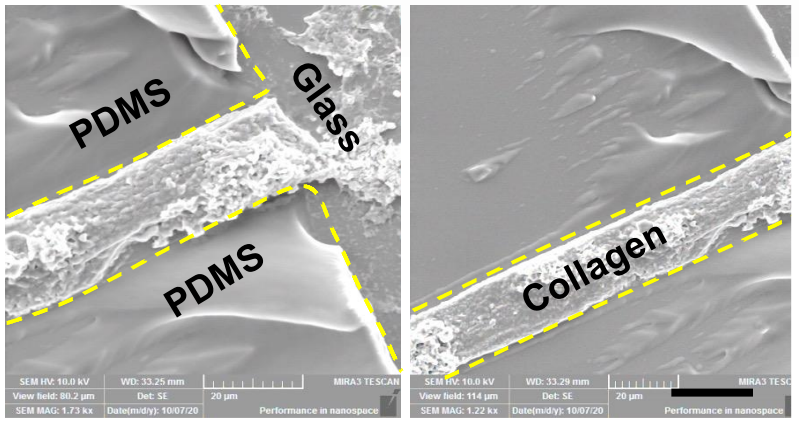
Microchannel



Functions

1) Localized cell loading
2) Cell migration
3) Biochemical diffusion
4) Mimic basement membrane with collagen
5) Imaging cellular interaction better than vertical type MPS model

Bright field microscopic image

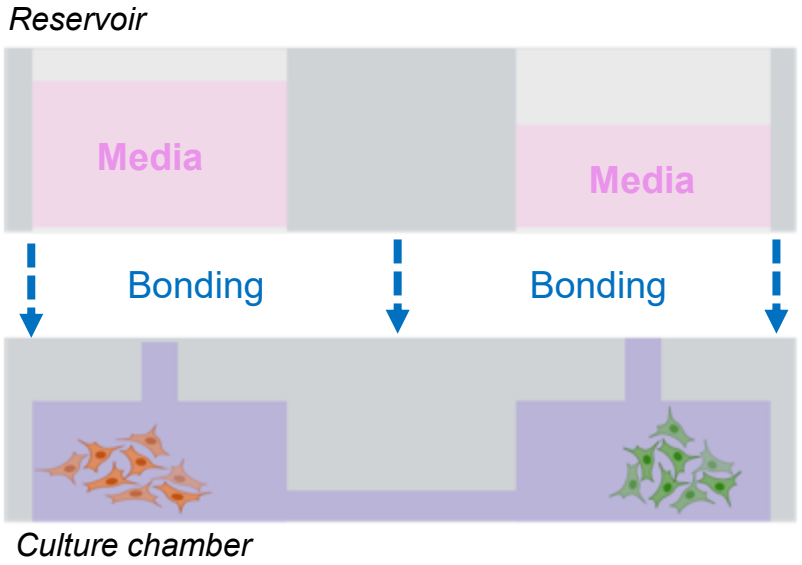


SEM image of microchannel

**Scale bar = 200 μm*

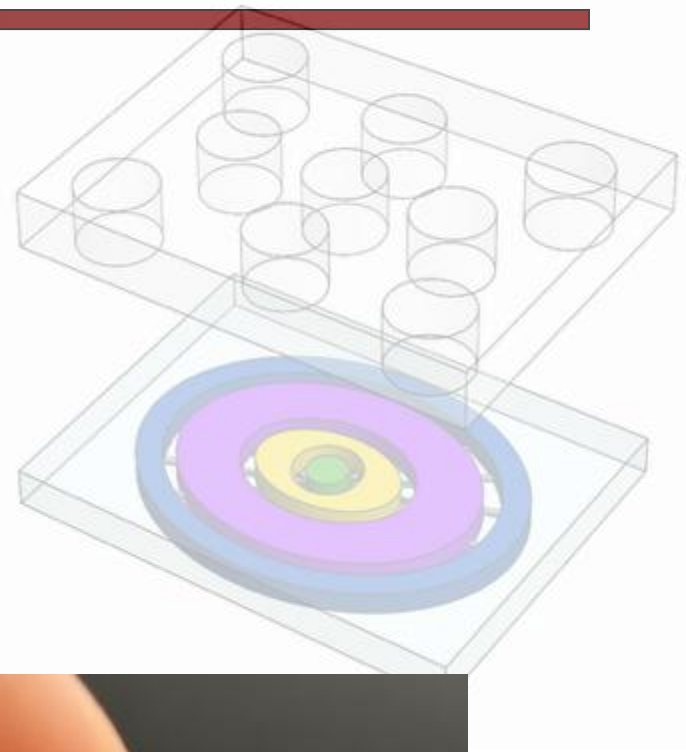
Our MPS Model Key features (2)

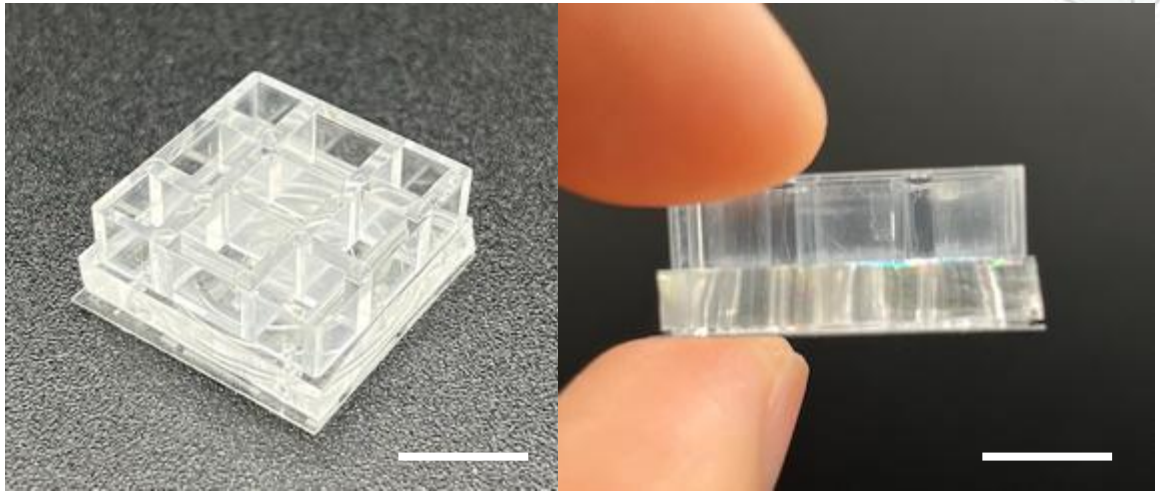
Media Reservoir block



Functions

1) Gradient-driven media diffusion
2) Enable localized media supply
3) Localized effluent collection
4) Localized treatment





Injection molded Polycarbonate Reservoir

**Scale bar=1cm*

Cell Resources needed for MPS Models for Pregnancy and Women's Health

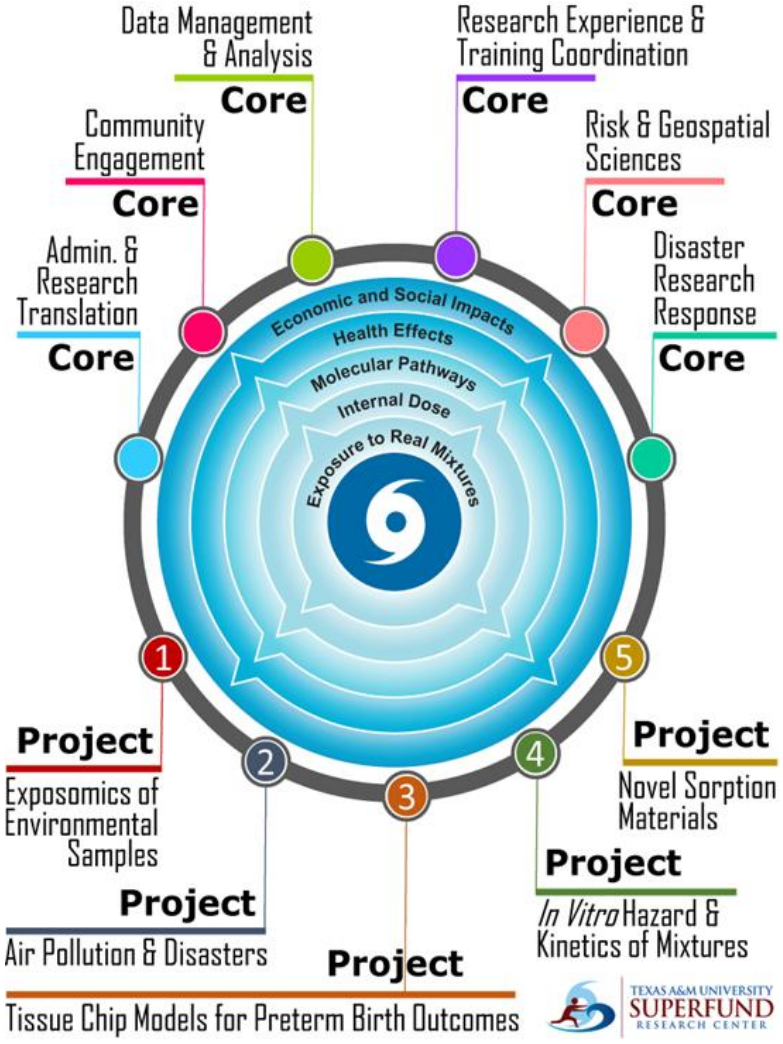
Cell types	AM-OOC	CDi-OOC	FMi-OOC	PLA-OOC	FMi-PLA-OOC	2nd Trimester PLA-OOC	VCD-OOC
Decidual cells (DEC)							
Chorion trophoblast cells (CTC)							
Syncytiotrophoblasts cells (STB)							
Cytotrophoblast cells (CTB)							
Human umbilical cord endothelial cells (HUVEC)							
Amnion mesenchymal cells (AMC)							
Amnion epithelial cells (AEC)							
Endo cervical epithelial cells (ENDO)							
Ecto cervical epithelial cells (ECTO)							
Transition zone with ECTO & ENDO cells							
Cervical stromal cells (CSC)							
Vaginal epithelial cells (VEC)							
Placental stromal cell (STR)							

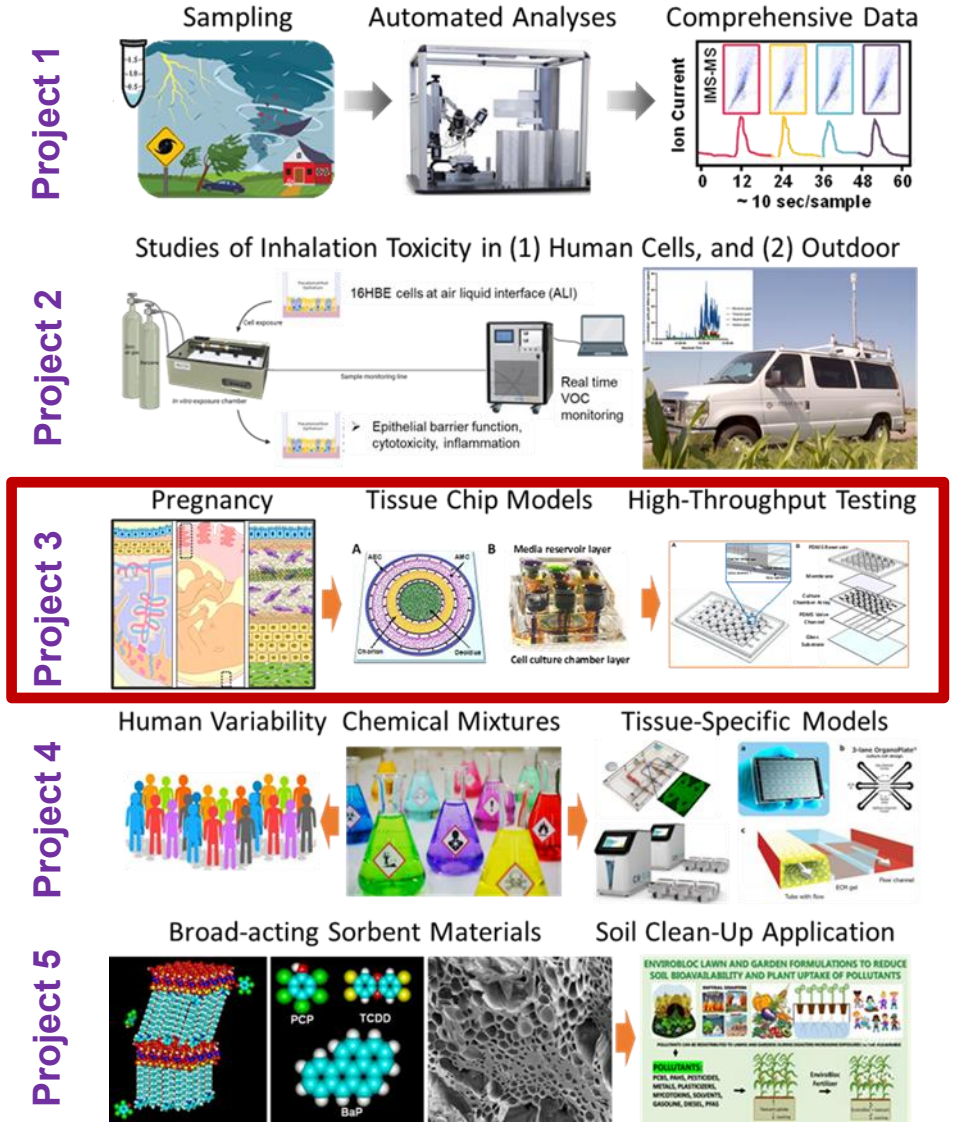
- **All 12 cell types (many from multiple individuals) are available as well-validated immortalized resources from Dr. Menon's Lab (UTMB)**
- **Tested through STR (Short Tandem Repeat) analysis, Karyotyping, RNA sequencing, Signaling pathway analysis, morphology analysis (immunostaining)**

PMID: 34935931

Texas A&M Superfund Research Center [2022-2027]

Comprehensive tools and models for addressing exposure to mixtures during environmental emergency-related contamination events



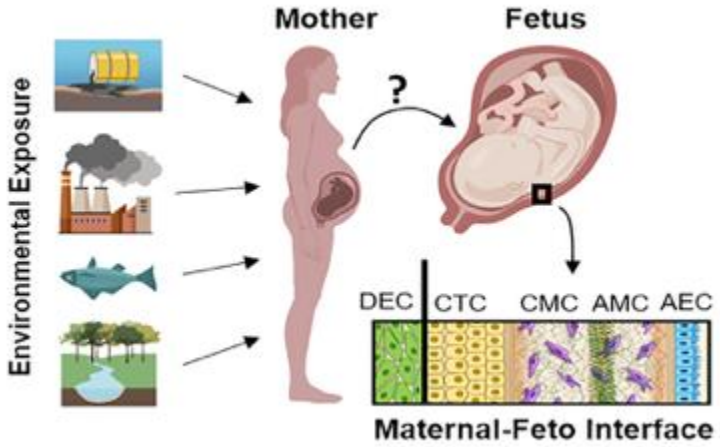


"One-Center" Approach to DR2

NIEHS P42 ES027704

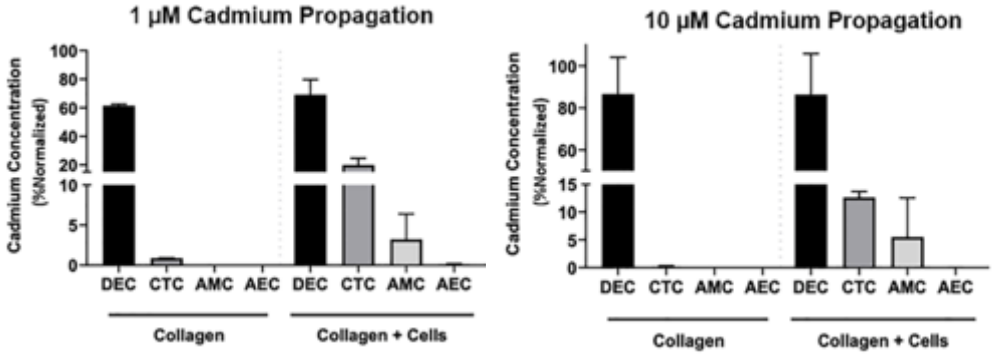
Using FMi-OOC for Toxicology and Toxicokinetic Studies

A. Maternal exposure to cadmium (Cd)

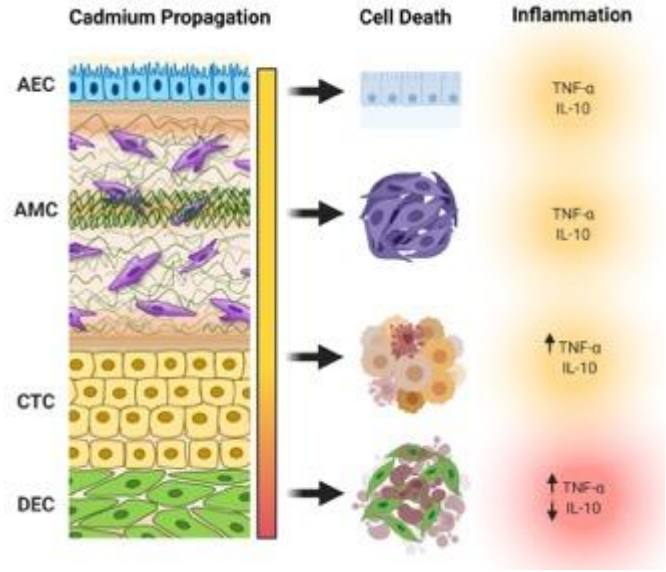


B. Toxicokinetic study with FMi-OOC

		DEC	CTC	AMC	AEC
Collagen	1 μM	0.6±0.007 μM	0.01±0.00005 μM	0±0 μM	0±0 μM
	10 μM	8.7±1.2 μM	0.02±0.001 μM	0±0 μM	0±0 μM
Collagen + Cells	1 μM	0.7±0.08 μM	0.2±0.04 μM	0.03±0.03 μM	0.001±0.0006 μM
	10 μM	8.6±1.6 μM	1.3±0.09 μM	0.6±0.6 μM	0.003±0.002 μM



C. Inflammatory responses



A. Exposure to environmental chemicals can trigger labor-initiating signals at FMi, leading to spontaneous preterm birth (PTB), however lack of studies and *in vitro* systems make this challenging

B. Cd transportation study using FMi-OOC showed cell-driven, limited transportation kinetics (DEC -> CTC) within FMi-OOC

C. Cd treatment resulted in significant cell death and pro-inflammatory responses in maternal decidua but minimal effect on fetal chorion cells, and no effect in the fetal amnion cells

➢ **FMi-OOC can assess chemical transport kinetics & toxicant exposure-mediated adverse effect at the FMi**

Kim et al., Journal of Hazardous Materials, 2022 (PMID: 34391970)

Microphysiological system (MPS)

3D human culture models; MPS; Organ-on-Chip; Tissue Chip

Can we use them as a routine tool/model?

Unique Features

More *in vivo*-like *in vitro* system

Control of microenvironment

Multiple cell types in 3D

Microscopy-compatible

Can be integrated with sensors

Challenges

Requires (often times) dedicated tool & instrument

Poor workflow compatibility

Poor user-friendliness

Not high throughput

Microphysiological system (MPS)

3D human culture models; MPS; Organ-on-Chip; Tissue Chip

Can we use them as a routine tool/model?

Unique Features

More *in vivo*-like *in vitro* system

Control of microenvironment

Multiple cell types in 3D

Microscopy-compatible

Can be integrated with sensors

Challenges

Requires (often times) dedicated tool & instrument

Poor workflow compatibility

Poor user-friendliness

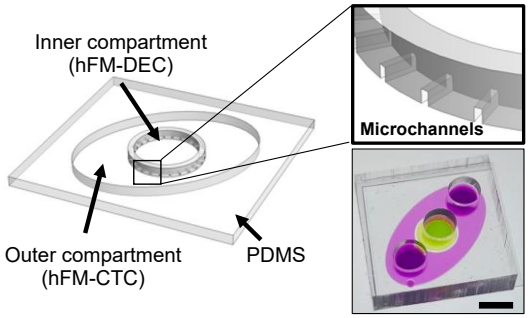
Not high throughput

Can we improve this?

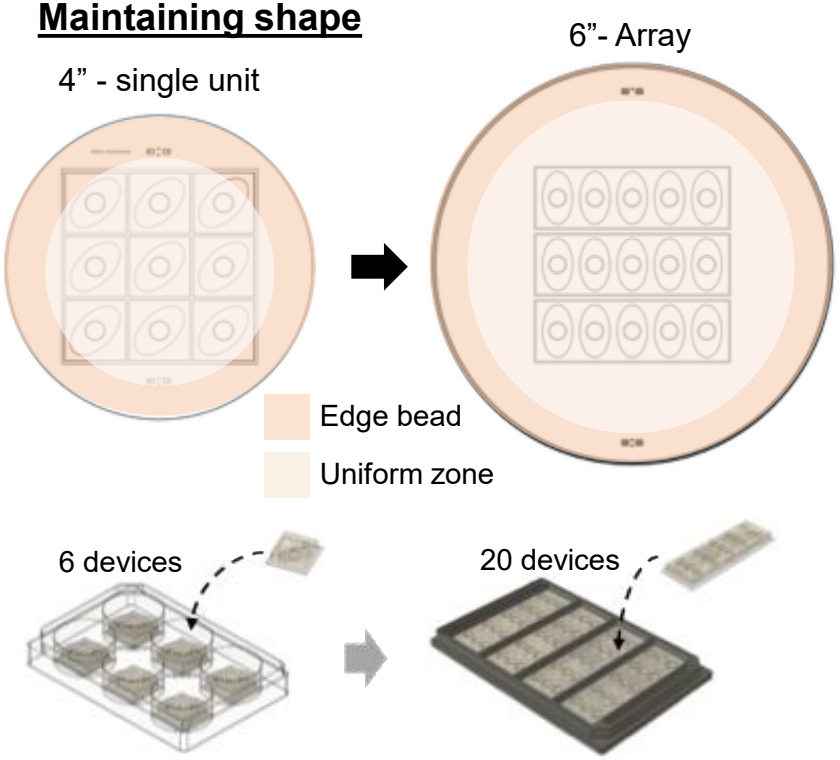
Increasing Throughput to Improve MPS Utility

A

Two chamber OOC (CD-OOC)

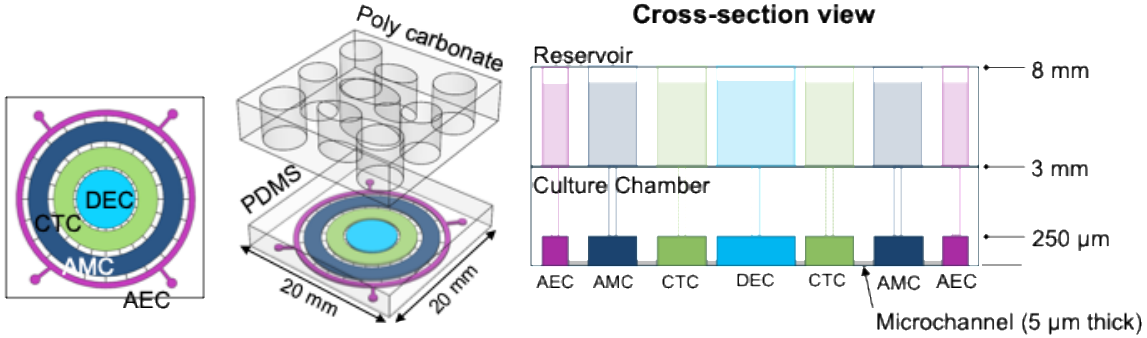


Maintaining shape

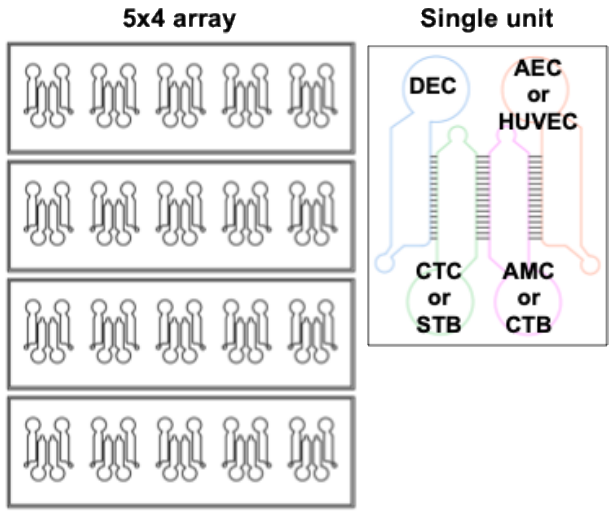


B

Four chamber OOC (FMi-OOC)



Modification for high throughput

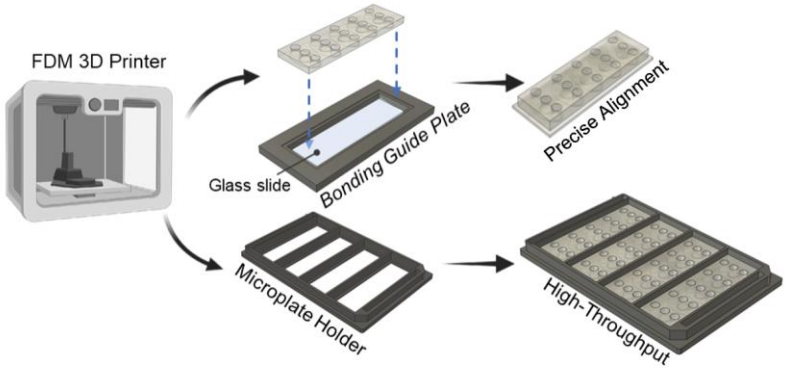


A. *Operational improvement - device configuration is maintained while adopting it to convenient/standard plate for high throughput operation

B. Design optimization – geometry can be modified for rapid assessment while maintaining functionalities, biocompatibility, and fluidic dynamics

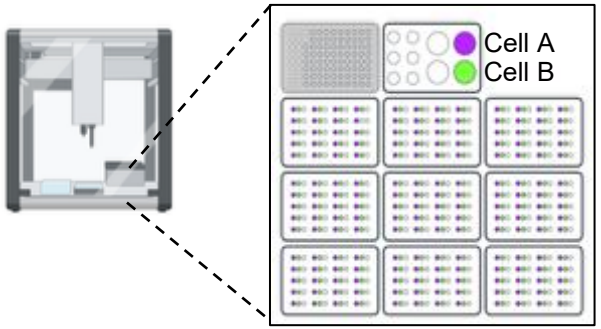
Adapting to Automated Testing Workflows

A 3D printing technology

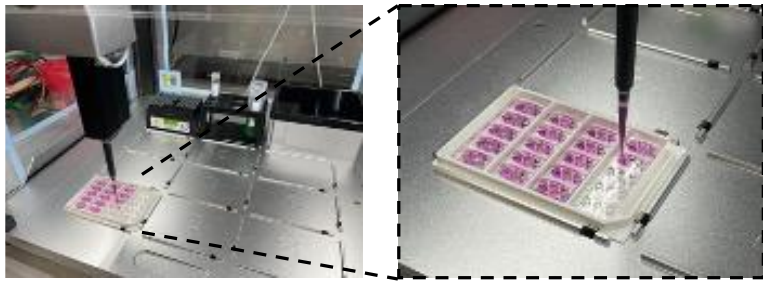


- Robust/firm printing for standard size of microplate (compatible with general microscopic station)

B Robotic liquid handler



180 MPS devices

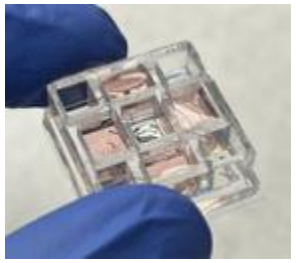


- Automatic liquid handling system (or multi-channel pipettors) support rapid and high throughput usage

C Injection molding technology and optimization process

PDMS reservoir

Poly styrene (PS) reservoir

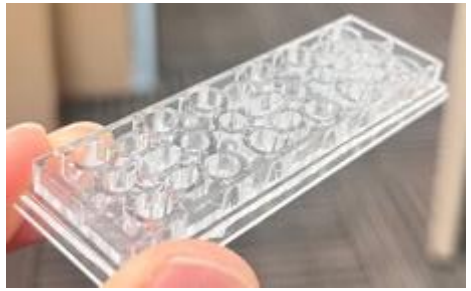


PC reservoir

Design of arrayed reservoir

Actual design for manufacturing

Final reservoir



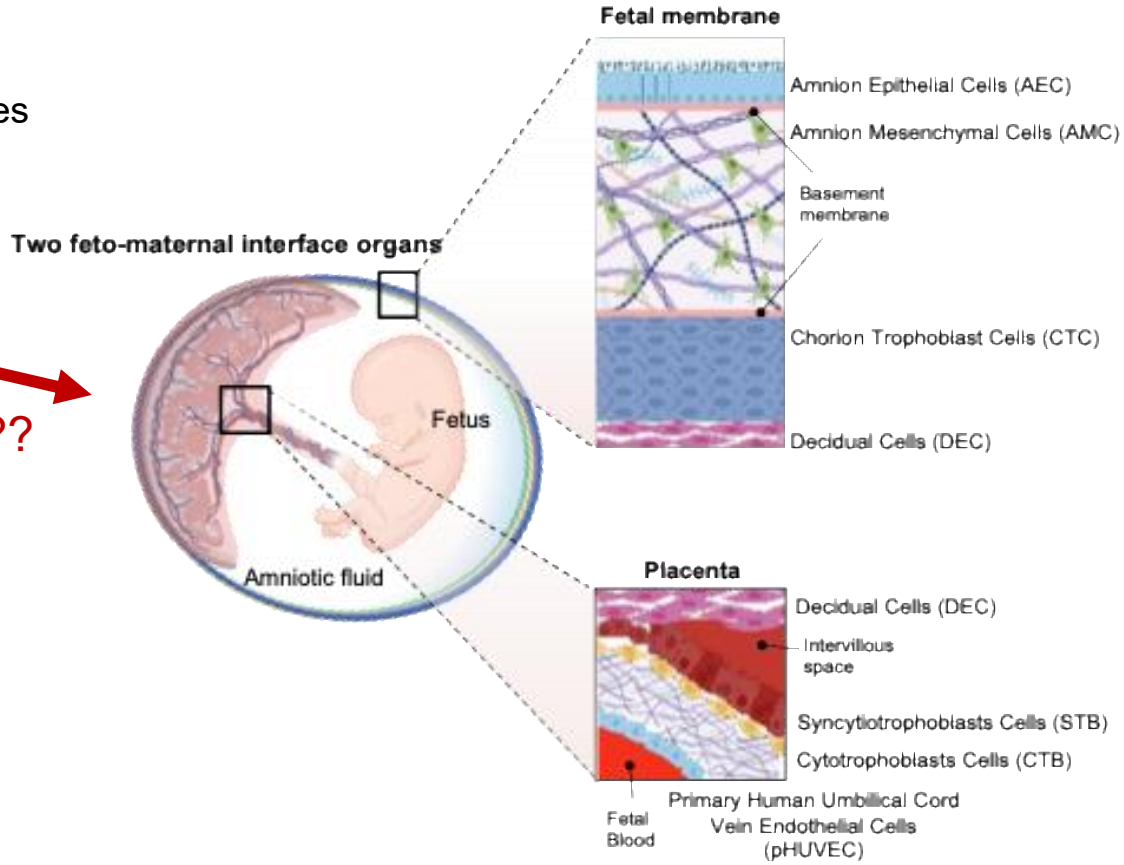
- Injection molding of thermoplastic guarantees consistent quality of platform development
- Currently utilizing on-going projects

Lam et al., Micro and Nano Systems Letters, 2025 (PMID: 41480333)

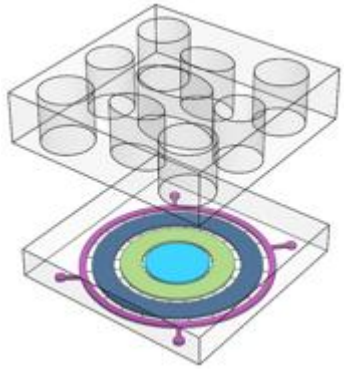
Using Pregnancy MPS Models to Study Hazardous Chemicals

Environmental Hazardous Substances

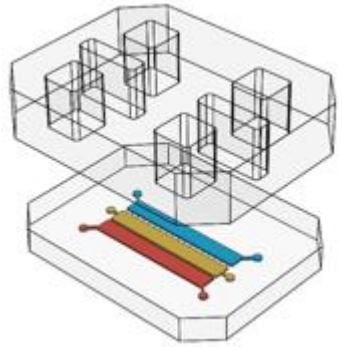
??



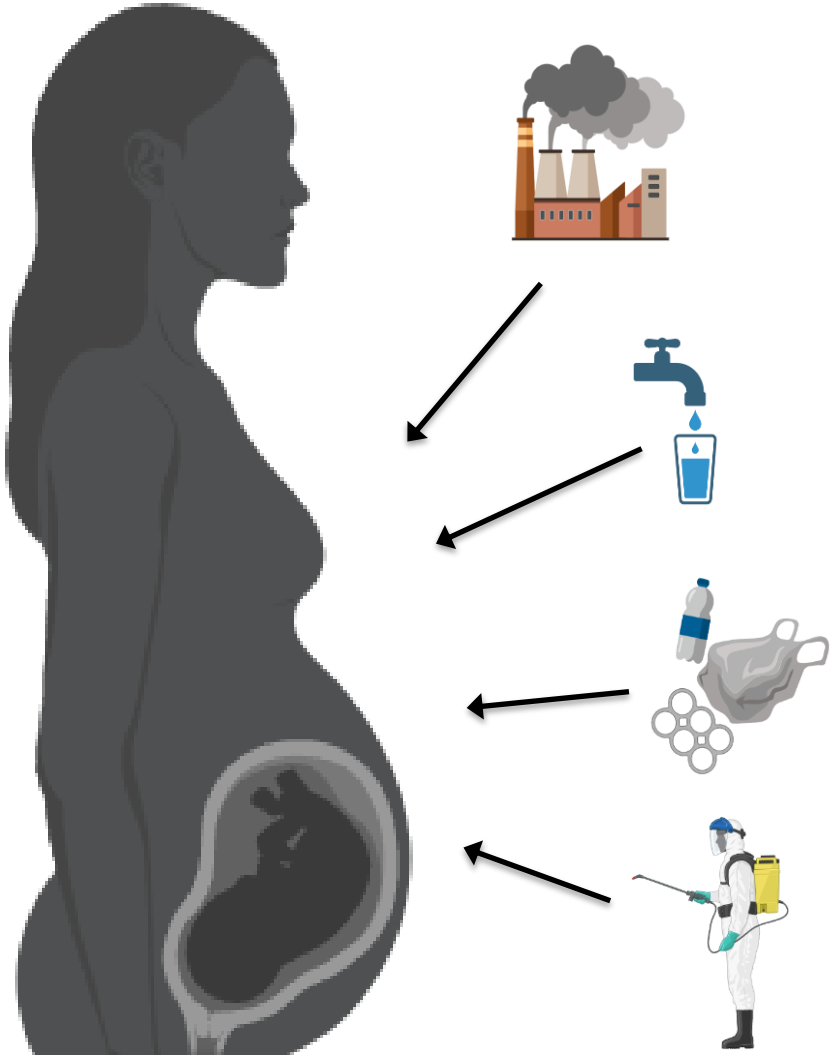
Fetal Membrane OOC



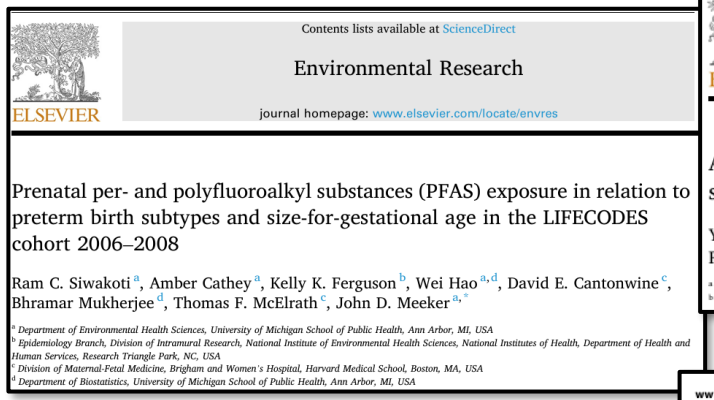
Placenta OOC



Using Pregnancy MPS Models to Study Hazardous Chemicals



- We are exposed to environmental toxicants every day from different sources
- Per- and polyfluoroalkyl substances (PFAS) are persistent chemicals, major public health concern, and may contribute to preterm birth



Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Prenatal per- and polyfluoroalkyl substances (PFAS) exposure in relation to preterm birth subtypes and size-for-gestational age in the LIFECODES cohort 2006–2008

Ram C. Siwakoti [a], Amber Cathey [a], Kelly K. Ferguson [b], Wei Hao [a,d], David E. Cantonwine [c], Bhramar Mukherjee [d], Thomas F. McElrath [c], John D. Meeker [a,*]

[a] Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI, USA
[b] Epidemiology Branch, Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC, USA
[c] Division of Maternal-Fetal Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[d] Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, MI, USA

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Association of prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances with fetal growth trajectories

Yi Yang [a,1], Sheng Teng [a,1], Leshi Lin [a], Wenjuan Li [a], Zhenzhen Zhu [a], Tangxin Chen [a], Li Li [b], Fang Peng [b], Dongmei Peng [b], Xiao Gao [a,*]

[a] Key Laboratory of Molecular Epidemiology of Hunan Province, Hunan Normal University School of Public Health, Changsha, 410081, China
[b] Changsha Hospital for Maternal & Child Health Care Affiliated to Hunan Normal University, Changsha, 410007, China

Contents lists available at ScienceDirect

Environmental Research

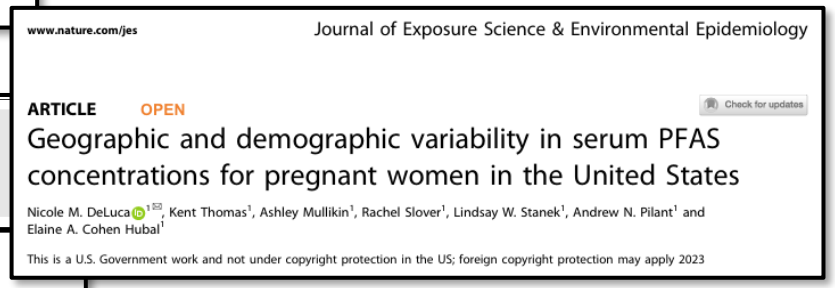
journal homepage: www.elsevier.com/locate/envres

Review article

Systematic review and meta-analysis of birth weight and PFNA exposures

J.M. Wright [a,*], A.L. Lee [a], K.M. Rappazzo [b], H. Ru [a], E.G. Radke [a], T.F. Bateson [a]

[a] US EPA, Office of Research and Development, Center for Public Health & Environmental Assessment, Chemical and Pollutant Assessment Division, USA
[b] US EPA, Office of Research and Development, Center for Public Health & Environmental Assessment, Public Health and Environmental Systems Division, USA



www.nature.com/jes

Journal of Exposure Science & Environmental Epidemiology

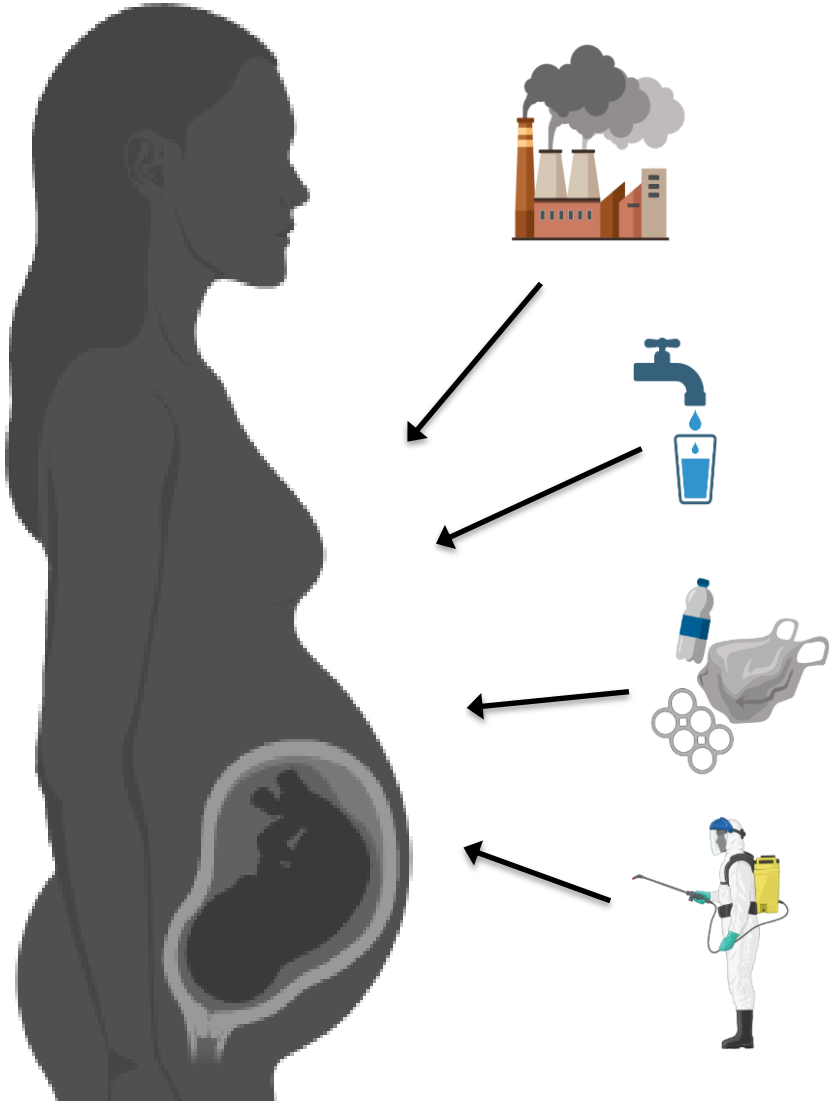
ARTICLE OPEN

Check for updates

Geographic and demographic variability in serum PFAS concentrations for pregnant women in the United States

Nicole M. DeLuca [1✉], Kent Thomas [1], Ashley Mullikin [1], Rachel Slover [1], Lindsay W. Stanek [1], Andrew N. Pilant [1] and Elaine A. Cohen Hubal [1]

Using Pregnancy MPS Models to Study Hazardous Chemicals

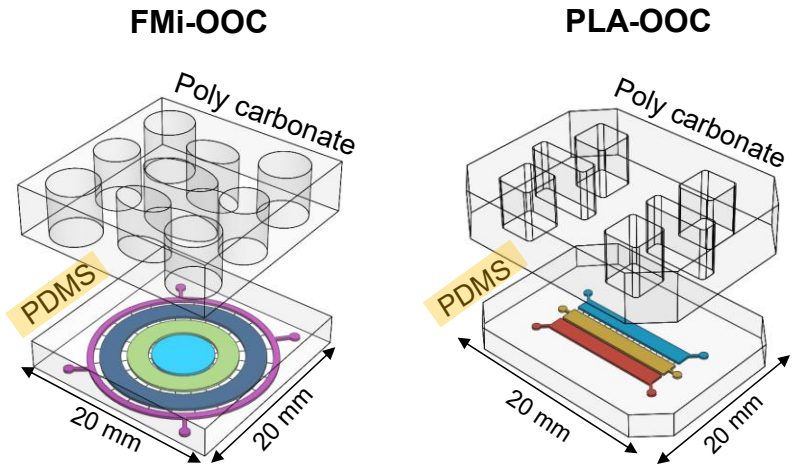


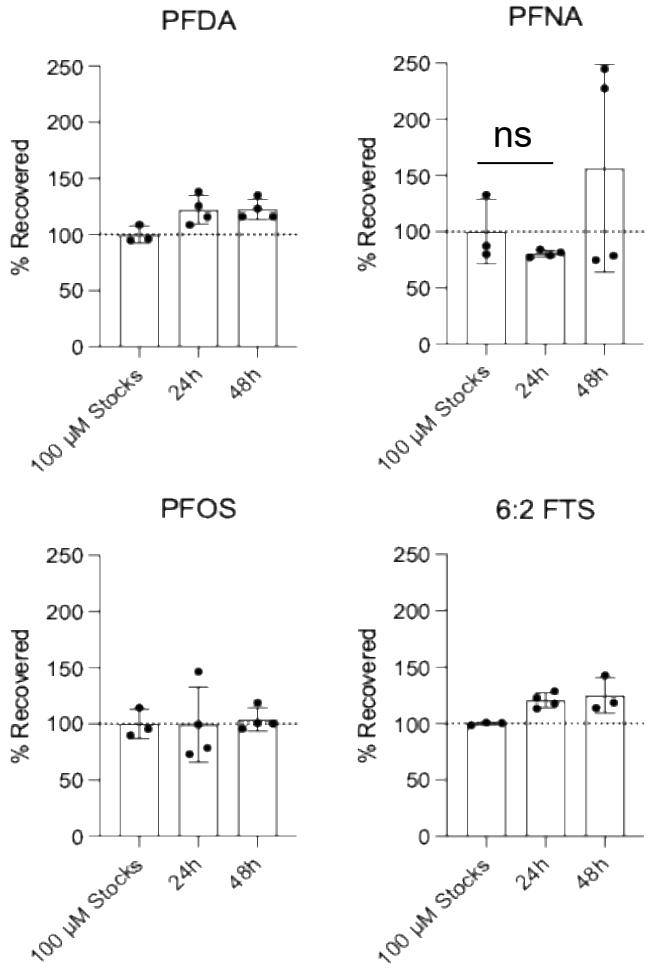
- **Hypothesis:** MPS models can be used to study environmental toxicants and their potential harmful effects on both the placenta and fetal membrane interfaces
- **Objective:** To evaluate individual PFAS chemicals (e.g., PFDA, PFNA, PFOS, 6:2 FTS) on placenta and fetal membrane under direct and indirect exposure using humanized *in vitro MPS* models

**Perfluorodecanoic acid (PFDA), Perfluorononanoic acid (PFNA), perfluorooctanesulfonate (PFOS), 6:2 Fluorotelomer Sulfonic Acid (6:2 FTS)*

Adsorption of PFAS to Device Surface

Molecular recovery rate from device

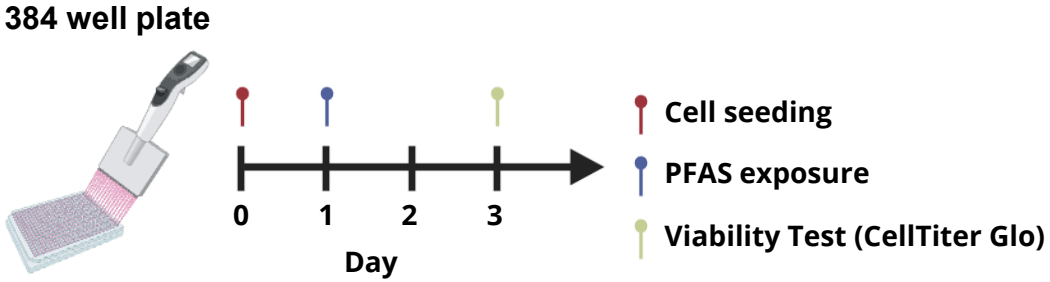




- Characterized adsorption of PFAS to device material
- Quantified recovery rate by LC-MS
- No adsorption was observed even after 48h
- **We can test these selected PFAS with our platforms**

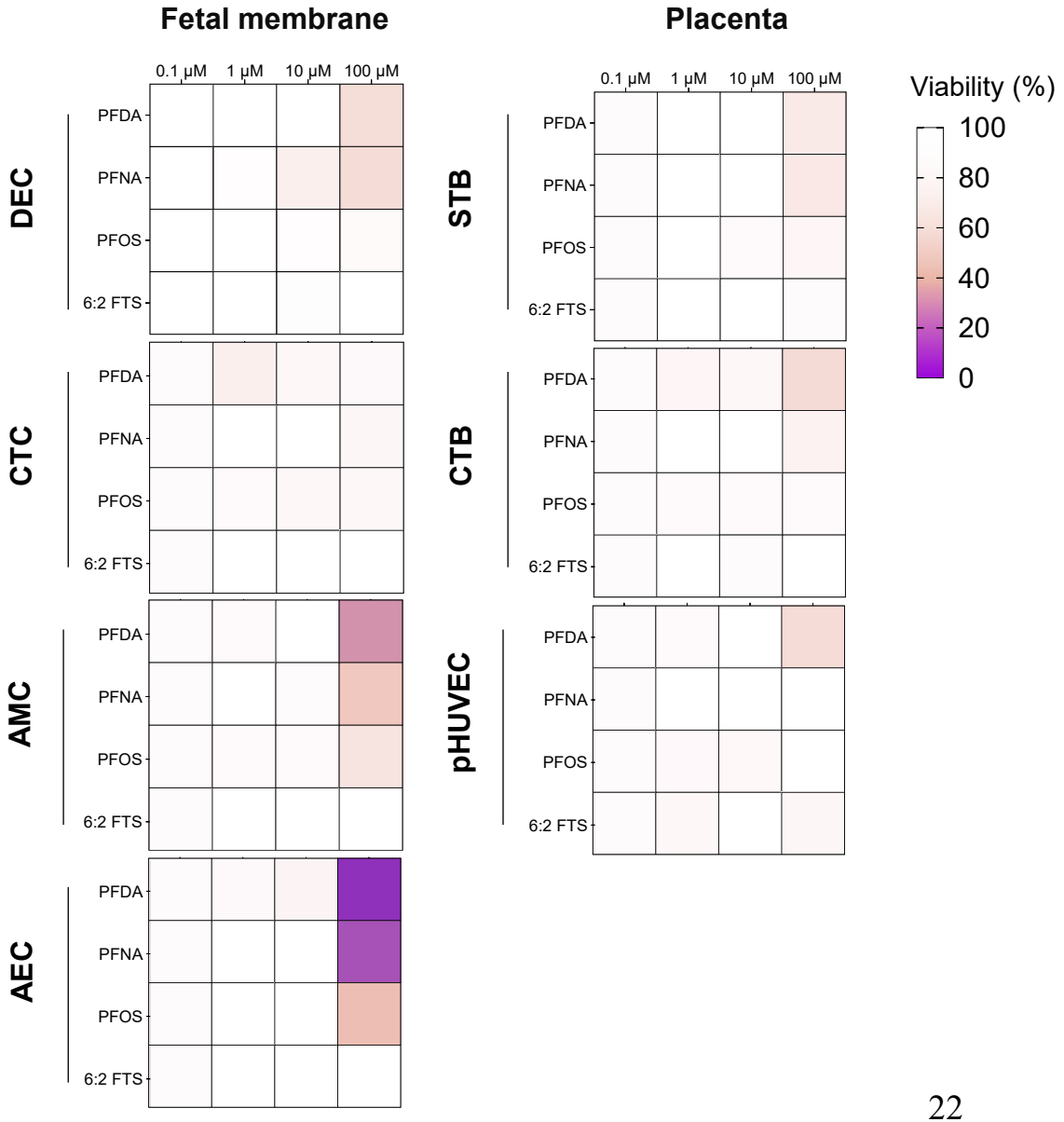
PFAS Direct Exposure in 2D for Dose Range Finding

Exposing to PFAS in 2D culture



- All cell lines were exposed with single PFAS individually
- With same cell density as in the MPS device
- Assessed viability after 48 h

- **Fetal membrane:** AEC and AMC were more responsive to PFAS, except 6:2 FTS
- **Placenta:** Cells were relatively more resistant to all PFAS chemicals

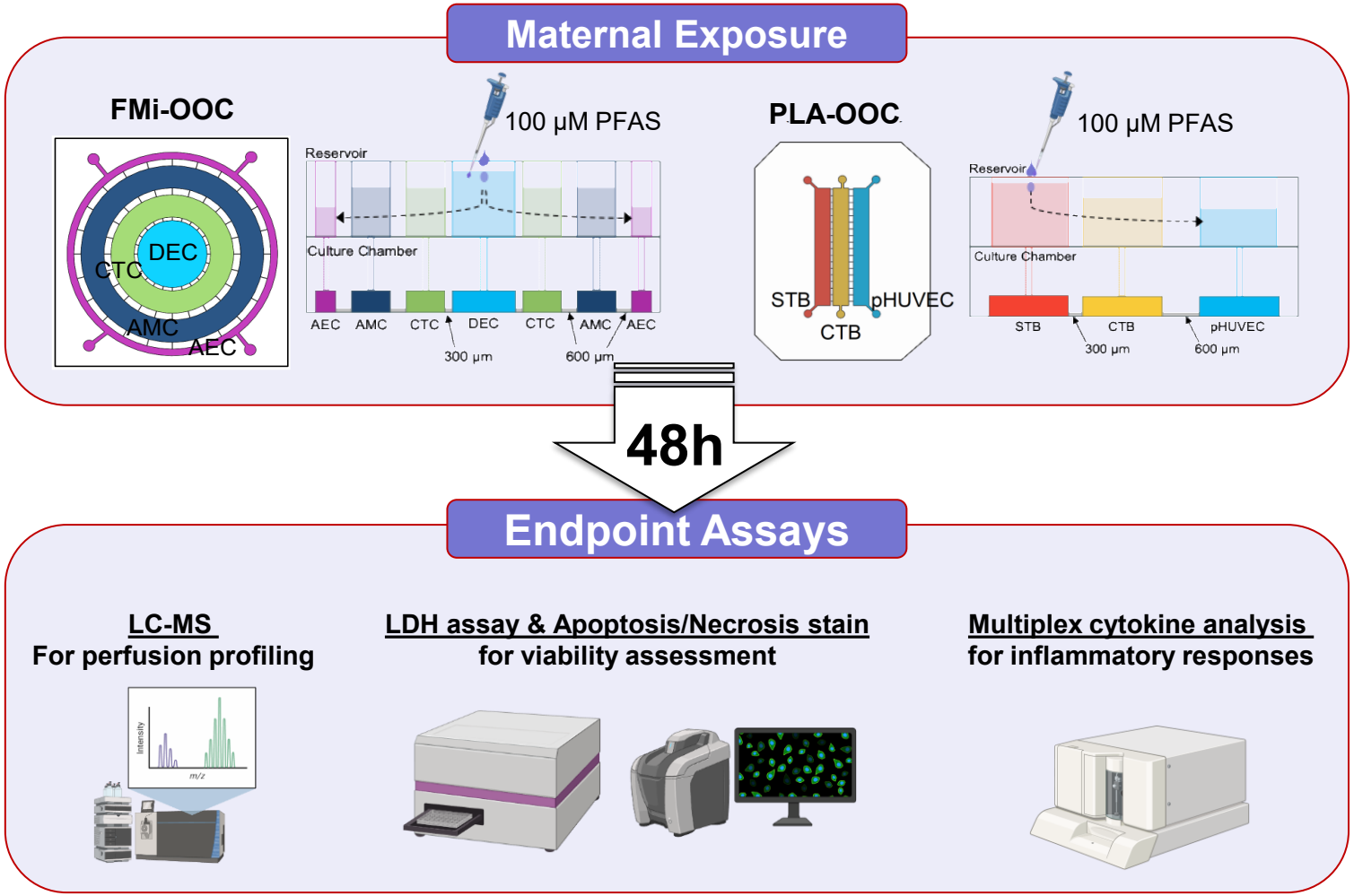


PFAS Study using Organ-on-Chip

Experimental Design Overview

- PFAS exposure was applied to maternal side of the MPS device
- Gravity-driven flow enhances directional perfusion to neighboring chambers
- Incubate for 48 h

- Effluents from each culture chamber and media reservoir collected and analyzed to assess cell-specific responses
- Image apoptotic/necrotic cells

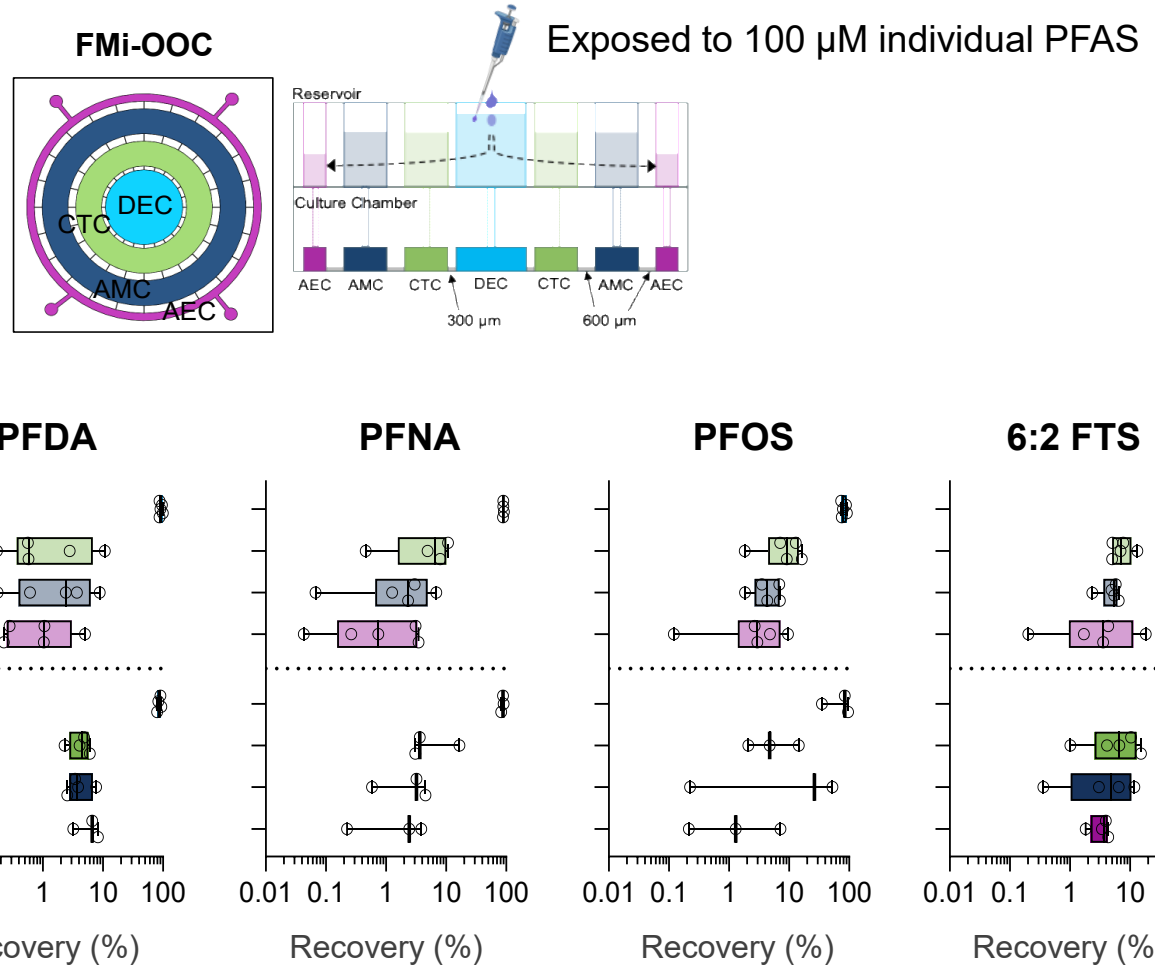


PFAS Study using Organ-on-Chip: Perfusion Profile

PFAS perfusion in FMi-OOC

Result

- PFAS propagated across the device and reached the fetal side (AEC, <10%)

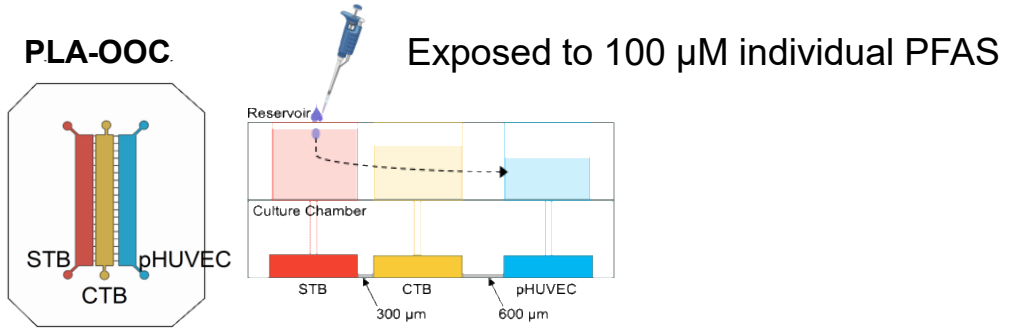


PFAS Study using Organ-on-Chip: Perfusion Profile

PFAS perfusion in PLA-OOC

Result

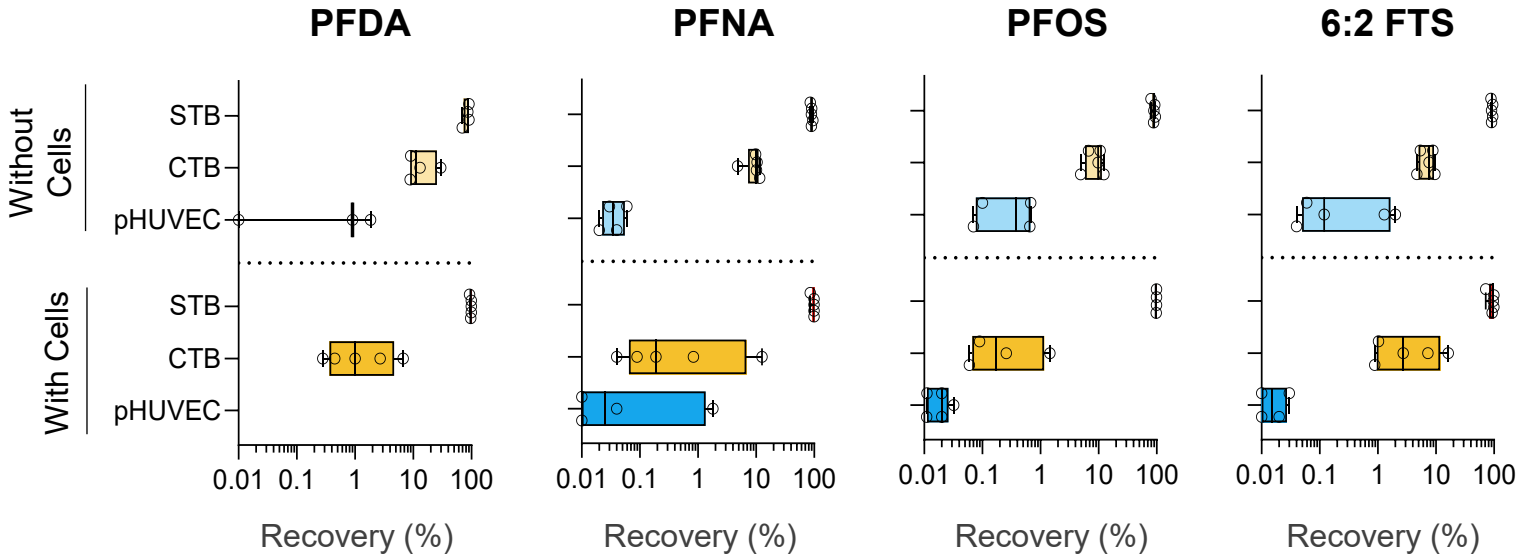
- Similar passive propagation was observed
- >90% PFAS was retained in the STB compartment



Exposed to 100 µM individual PFAS

Fetal membrane: Most PFAS remained on the maternal side; very limited diffusion to fetal cells

Placenta: Barrier strongly retained PFAS in the trophoblast compartment



PFAS Study using Organ-on-Chip: Viability Assessment

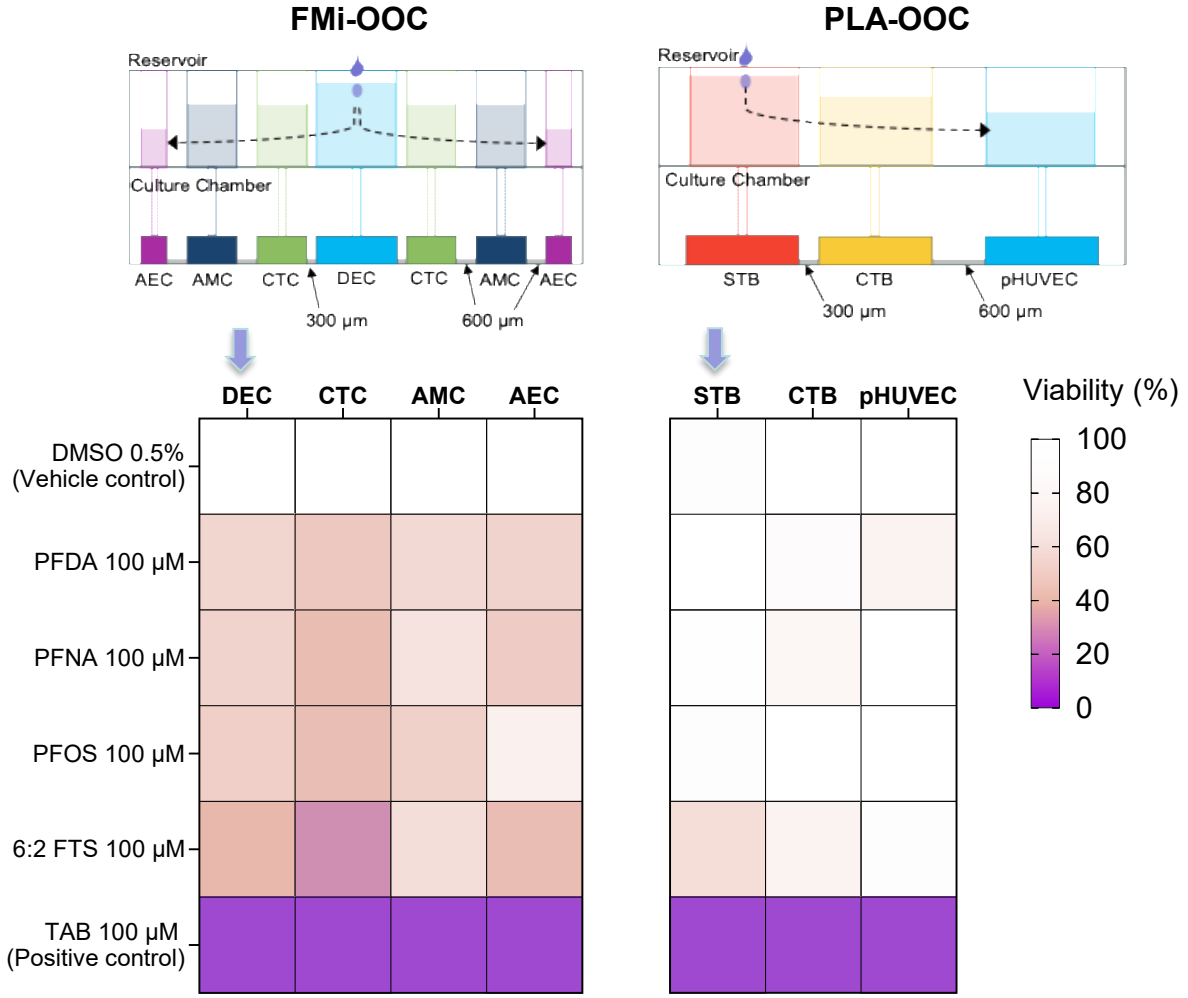
Viability assessment – LDH assay

Fetal membrane:

- Cells were actively responding
- DEC & CTC were more responsive than in 2D culture, indicating cellular interaction between each cell layer

Placenta:

- Cells showed a similar response to 2D (direct) exposure
- Indicating robust barrier integrity

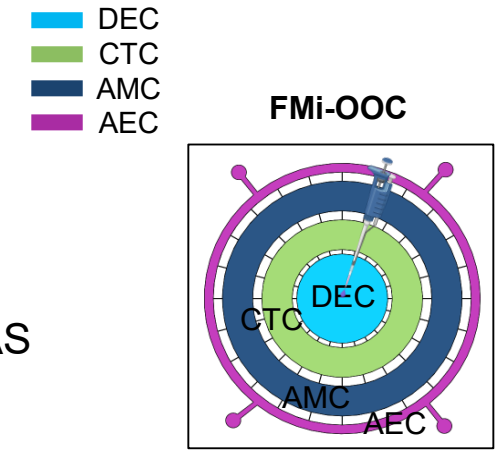


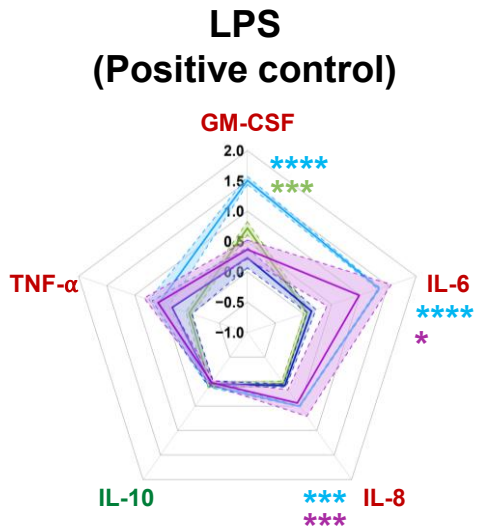
PFAS Study using Organ-on-Chip: Inflammatory Responses

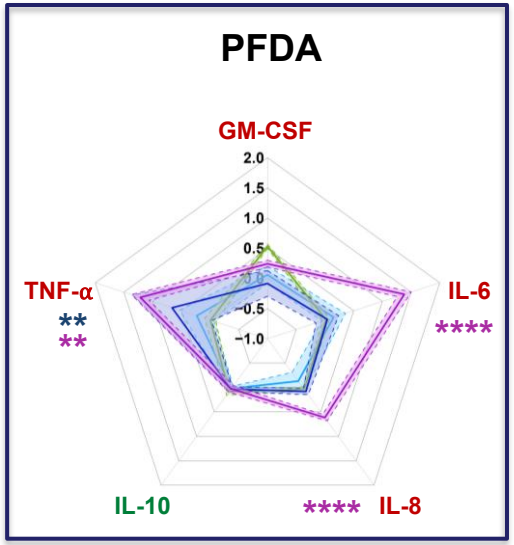
Cytokine analyses in FMi-OOC

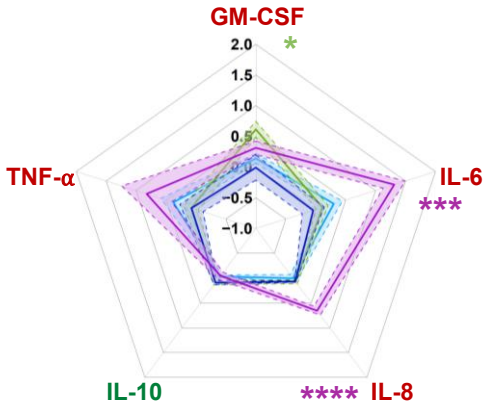
Result

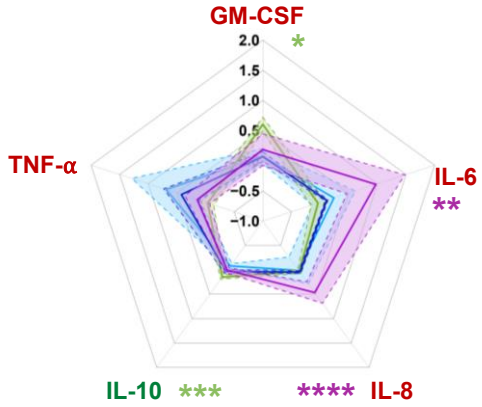
- Limited decidual response despite high PFAS presence
- PFDA induced the most significant fetal inflammation, especially in AEC, compared with the other PFAS

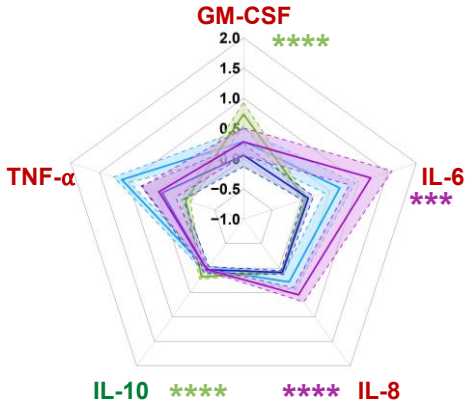










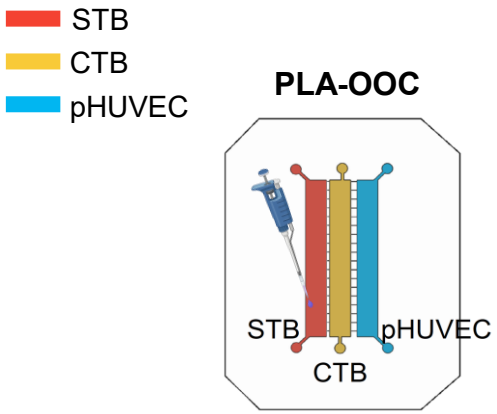


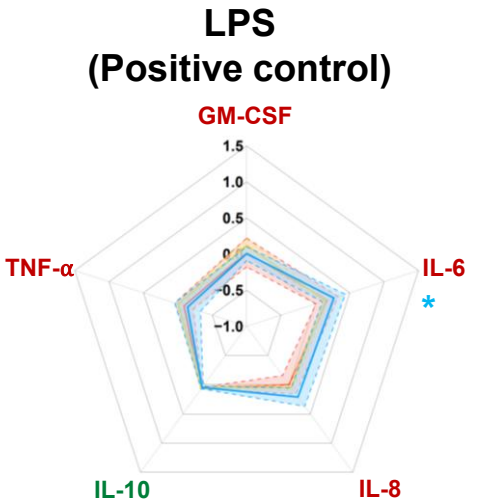
PFAS Study using Organ-on-Chip: Inflammatory Responses

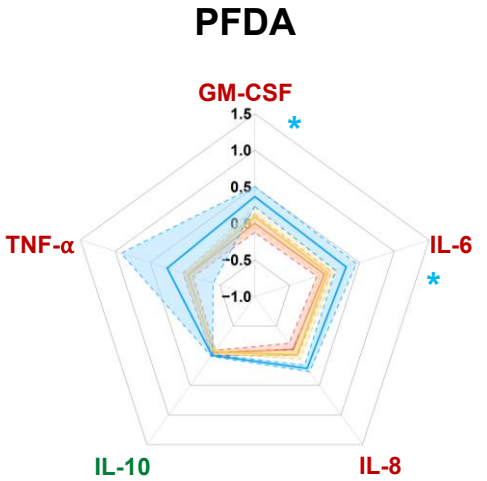
Cytokine analyses in PLA-OOC

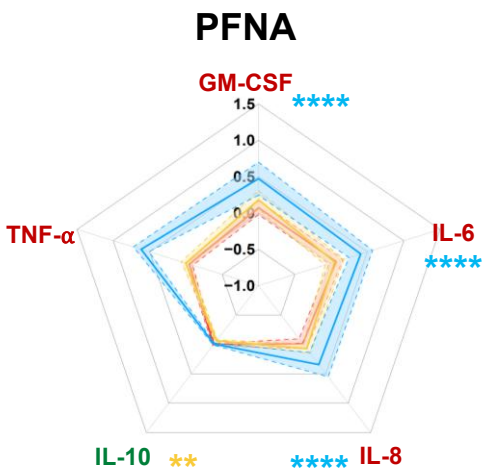
Result

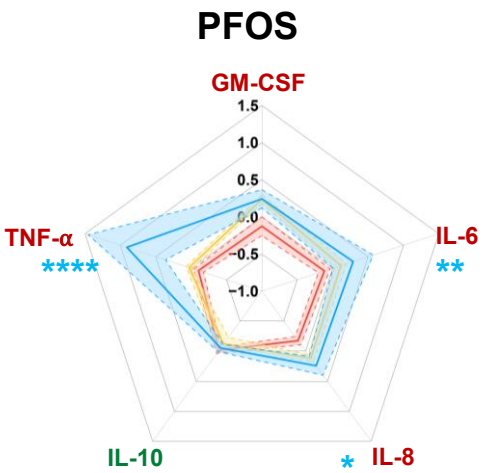
- STB & CTB showed high resistance to PFAS, with no observable inflammation
- pHUVEC demonstrated intercellular inflammatory responses

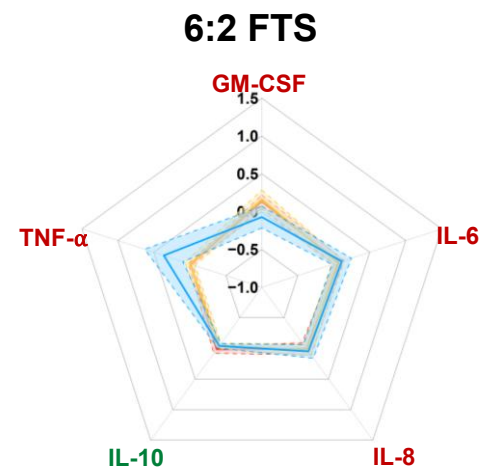






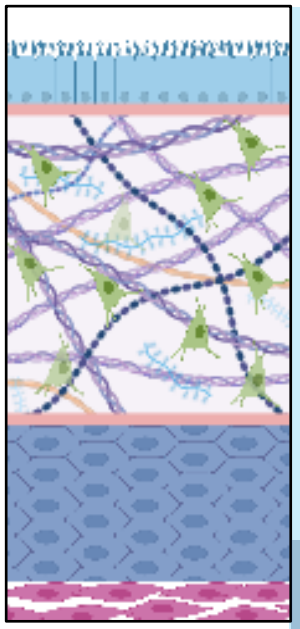






Different Responses of Fetal Membrane vs. Placental Interface

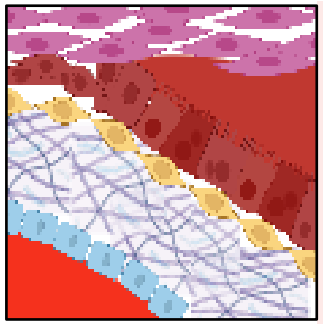
Fetal Membrane



- Direct exposure – only fetal AEC showed cytotoxic responses
- More reactive to PFAS (especially 6:2 FTS and PFDA/PFNA vs PFOS)
- Showed multicellular inflammatory and cytotoxic responses

✓ **Fetal membrane demonstrated multicellular, communicative inflammatory and cytotoxic responses, but these were not primarily driven by direct PFAS exposure**

Placenta



- Direct exposure – trophoblast cells and pHUVEC were resistant
- Robust barrier to PFAS (limited propagation)
- Trophoblasts resistant to cell death and inflammation
- pHUVECs demonstrated increased inflammatory responses, likely due to the multicellular culture

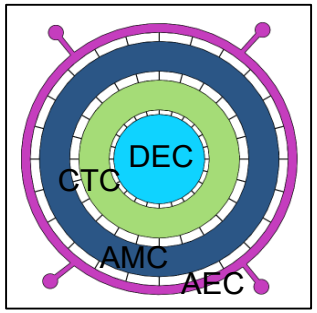
✓ **Robust integrity against PFAS exposure with respect to inflammation and cell death**

Summary

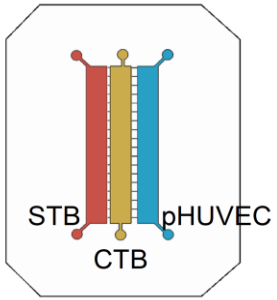
✓ **Conventional 2D experiment**

✓ **Baseline studies**

FMi-OOC



PLA-OOC



✓ **More physiological relevant microenvironment**

✓ **Pregnancy related NAMs for rapid screening**

- Both feto-maternal interfaces are important for maintaining pregnancy as protective tissues
- PFAS do not affect all feto-maternal tissues equally, depends on specific PFAS family chemical
- **Fetal membrane** showed both vulnerability (higher cytotoxic and inflammatory responses) and resiliency
- **Placenta** displayed robust barrier properties and resistance to PFAS-induced injury
- **Evaluating PFAS effects on both fetal membrane and placenta is essential**

Future Directions

- Further develop into **higher-throughput automated** NAMs models to have practical and routine utility in assessing potential hazards of environmental chemicals (ongoing and future TAMU Superfund Center Theme)
- Test **broad ranges** of PFAS chemicals (tens to hundreds)
- Test **individual chemical vs. mixture** (key TAMU Superfund Center Theme)
- Test **real-world samples from disaster** (key TAMU Superfund Center Theme)
- Establish as a **mechanistic tool** for studies of preterm birth
- Incorporate **population variability** into the MPS models (future TAMU Superfund Center Theme)

Acknowledgements

Collaborators:
Menon Laboratory (UTMB)
Rusyn Laboratory (TAMU)


Dr. Arum Han


Dr. Ramkumar Menon


Dr. Ivan Rusyn

Funding Sources:

- NIH-NIEHS-P42 ES027704 (Texas A&M Superfund Center)
- NIH-NIEHS-P30 ES029067
- NIH-NCATS-UH3 TR003283 (Clinical Trial on a Chip)
- NIH-NCATS-U2C TR004868 (TraCe MPS)
- NIH-NICHD-R01 HD100729

Lab Website:
https://nanobio.engr.tamu.edu/

ELECTRICAL & COMPUTER ENGINEERING
TEXAS A&M UNIVERSITY

NanoBio Systems Lab.

utmb Health

the menon laboratory

TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER