



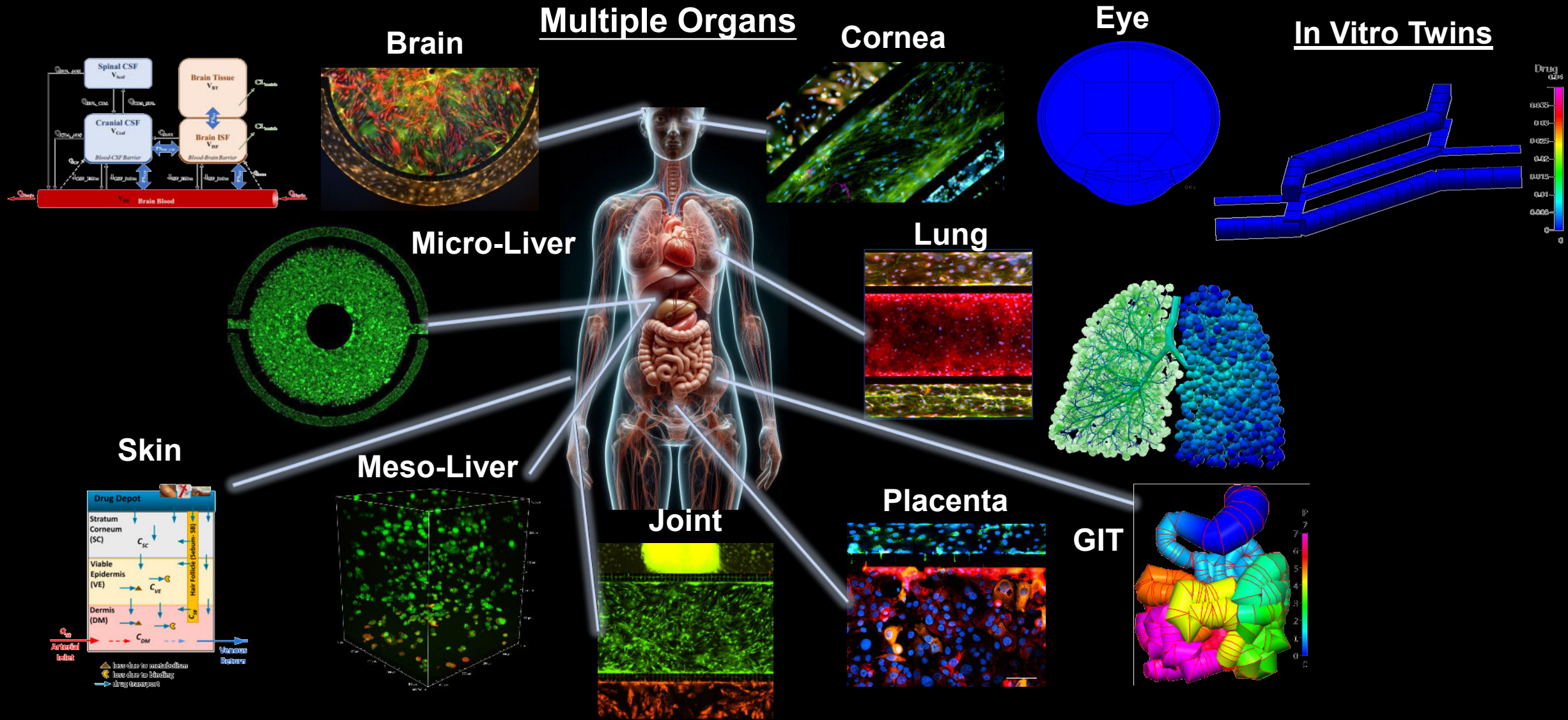
## An Organ-on-Chip Approach to Evaluation of Reproductive Health, Aging and Disease in Women

**PI:** Dr. Carrie German

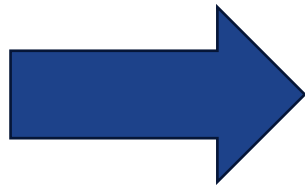
**Speakers:** Mr. Dylan Gordon and Dr. Ketan Bhatt

**Contract:** NIH NCATS TCIS 2.0 -1UG3TR005857-01

# in Vitro and in Silico Modeling at CFD Research



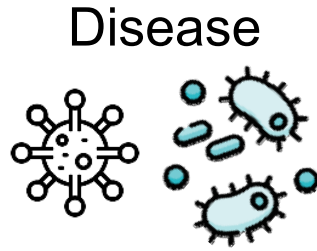
## Tissue Chips in Space 1.0



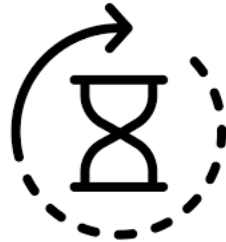
### Key Findings:

- Accelerated disease progression
- Accelerated aging

## Tissue Chips in Space 2.0

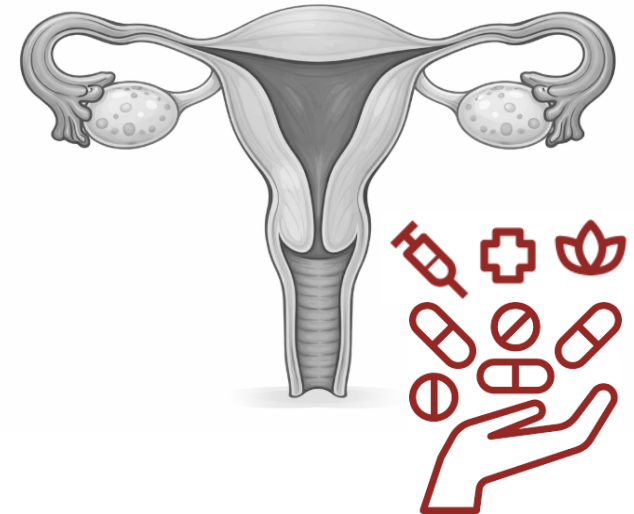


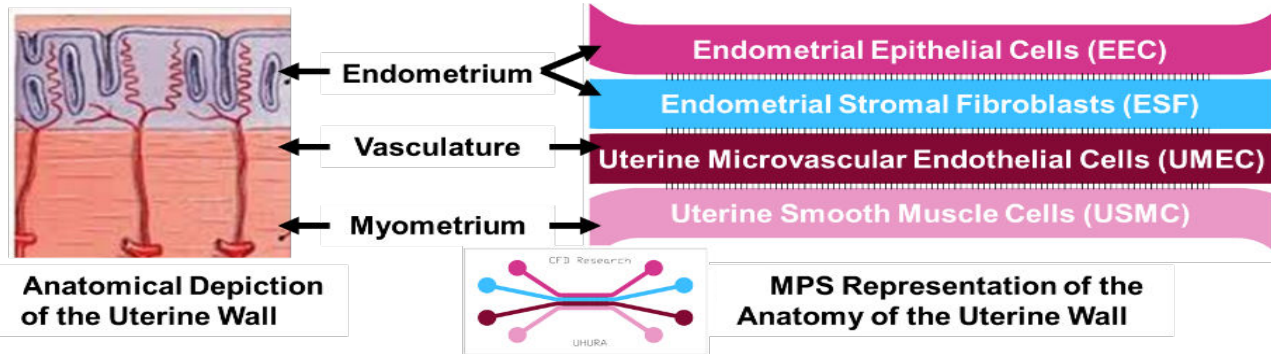
Aging



Astronaut Health

## Women's Reproductive Health (Uterine Aging)

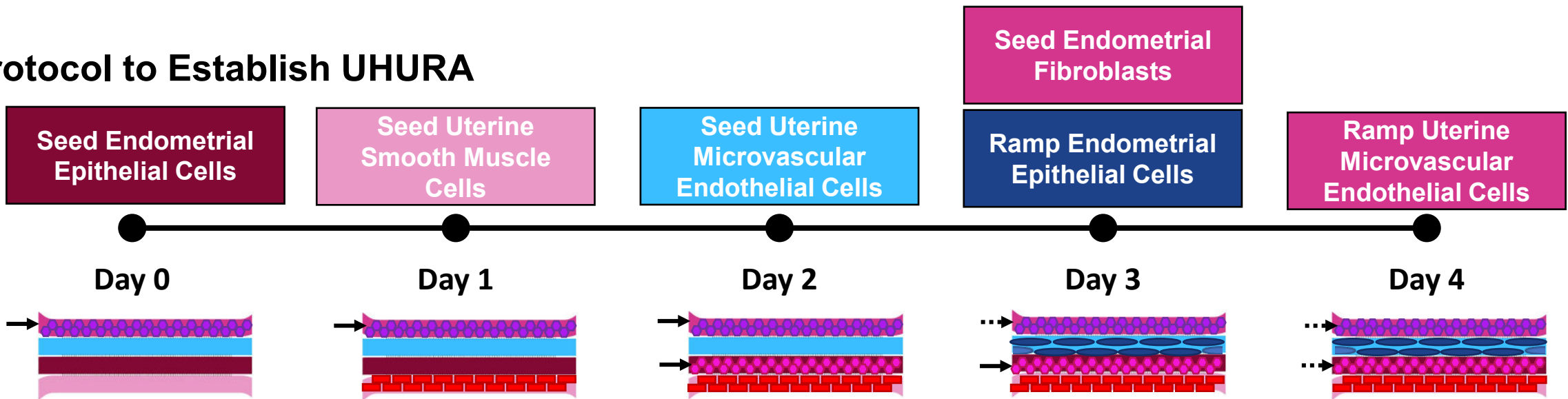




## Optimization

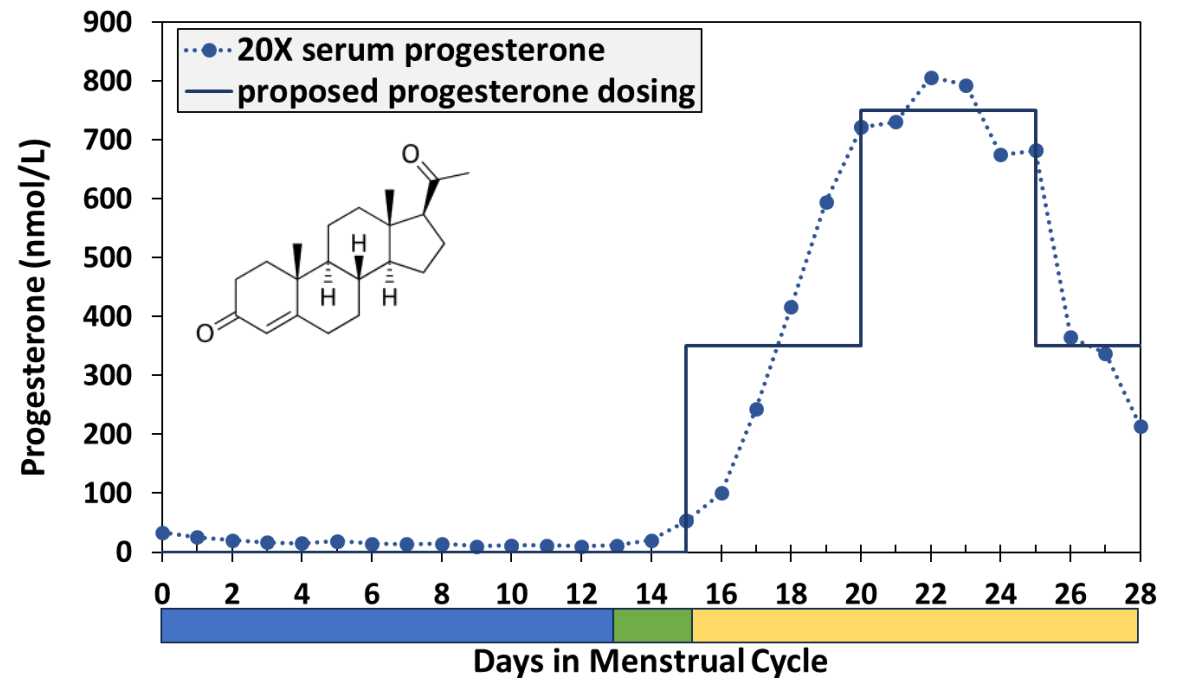
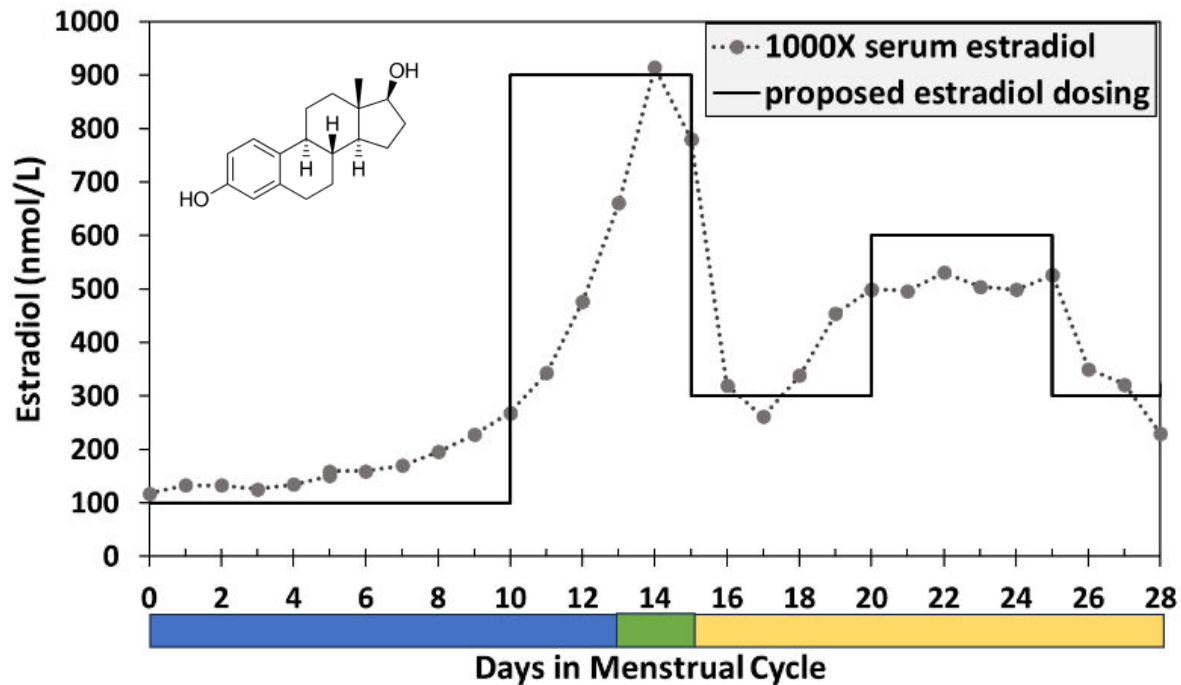
- ECM (per channel)
- Media composition
- Flow rates (per flow channel)
- Commercially available Primary cells (Lifeline & Promocell) used to establish
- USF provided cells for final model

## Protocol to Establish UHURA



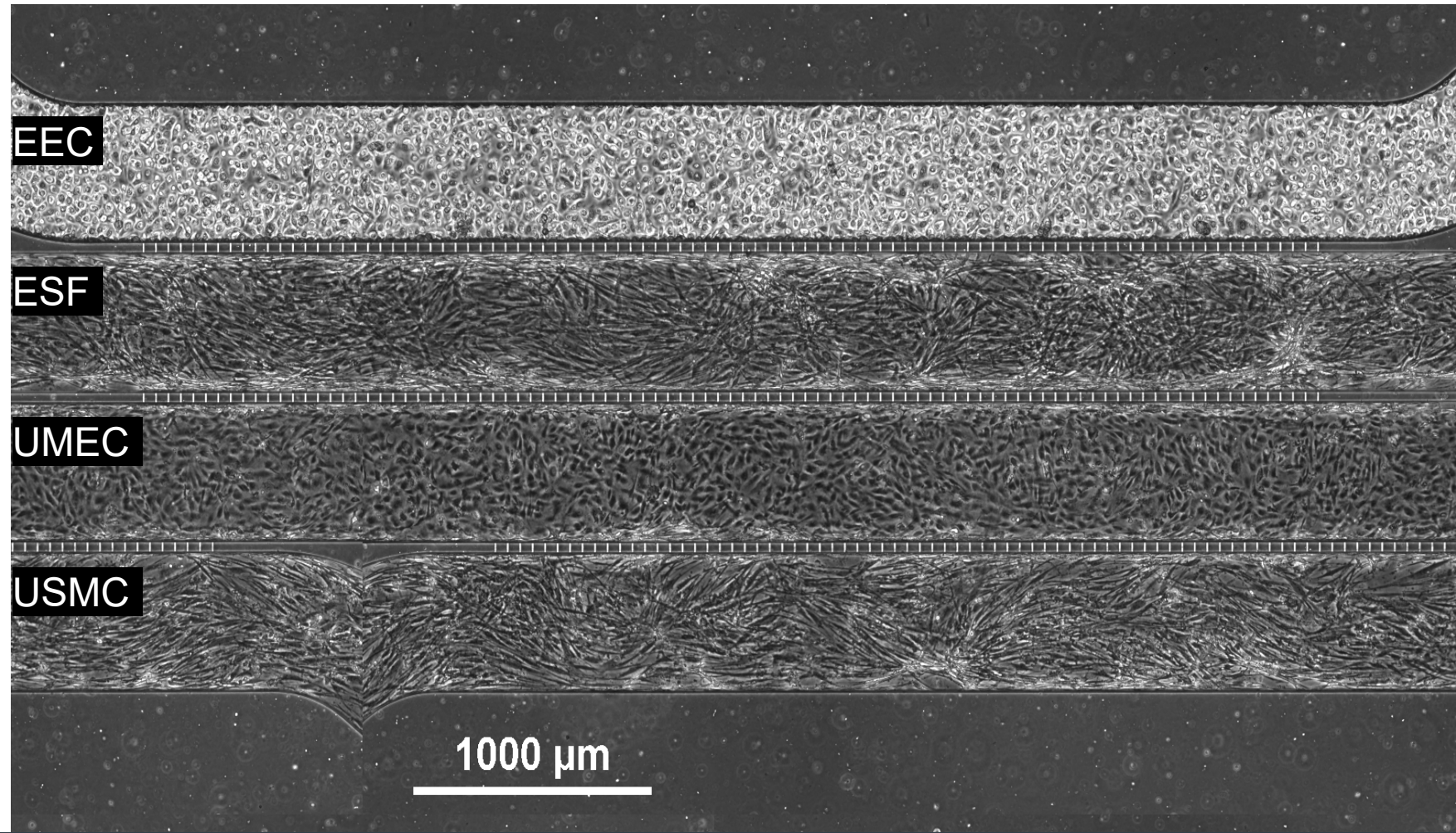
# Hormone Dosing Regimen

- Progesterone and Estrogen
- Introduced in media flow
- Values selected based on previously established in vitro values in literature

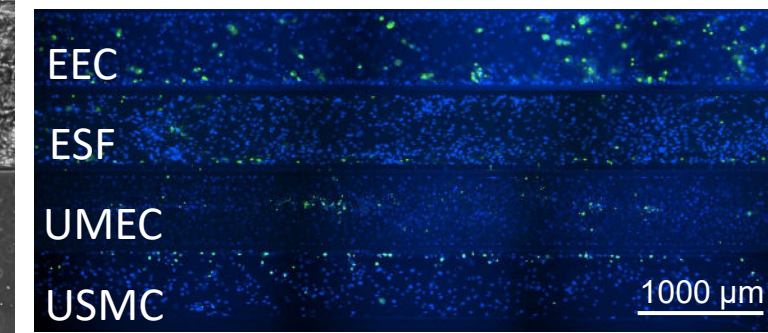
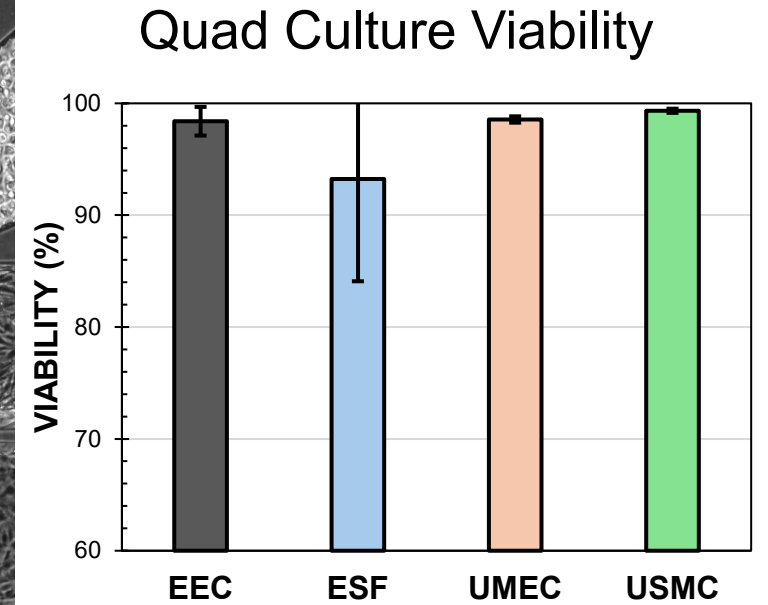




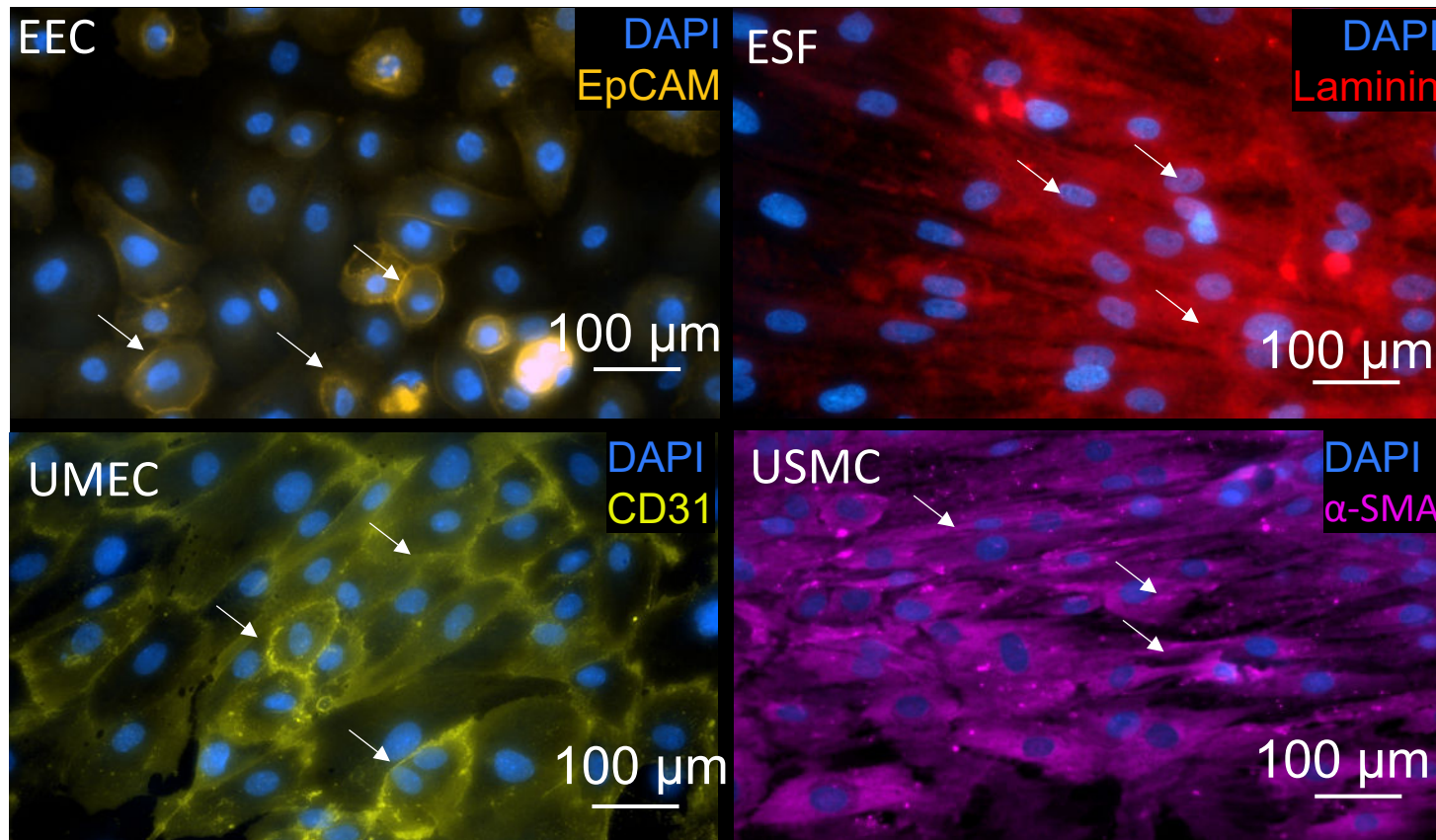
## Phase Microscopy – Uterine Quad Culture



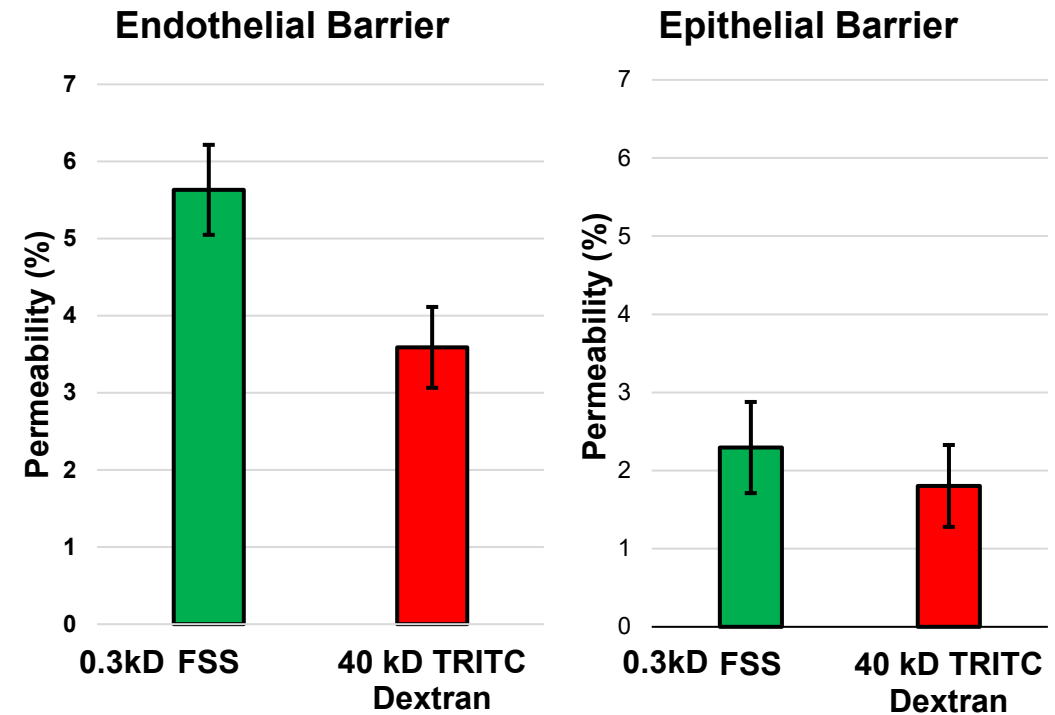
## Viability – Dead/All Staining



## Phenotype – ICC Staining



## Function – Permeability



## Model Establishment

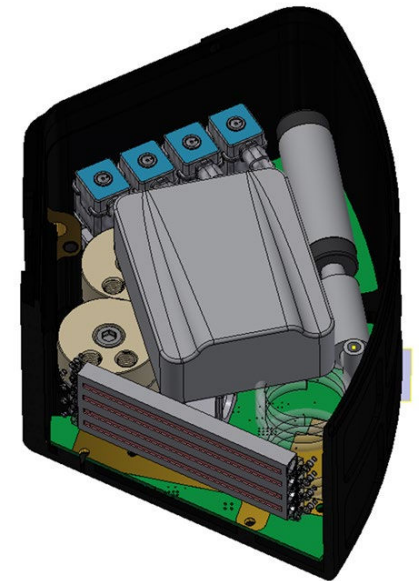
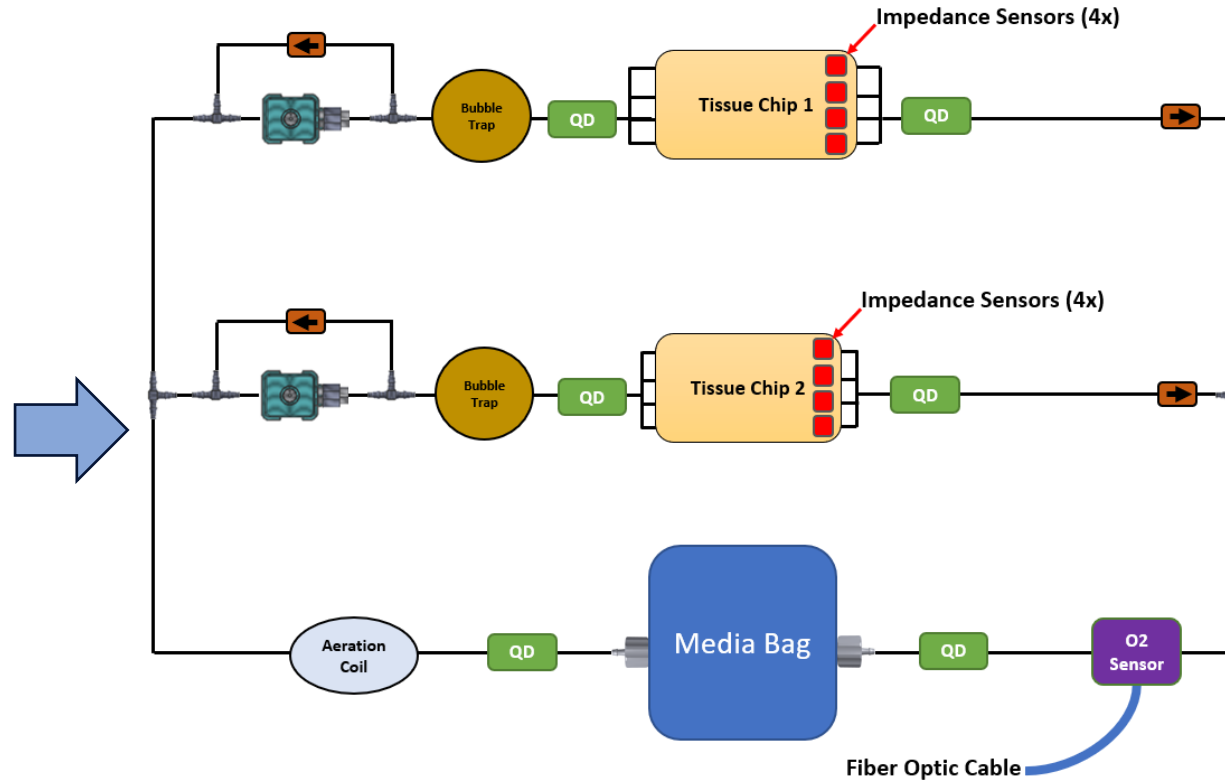
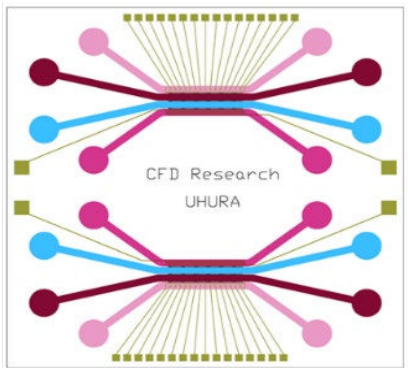
- ECM
- Media composition
- Flow Rates

## Cell Integrity Monitoring

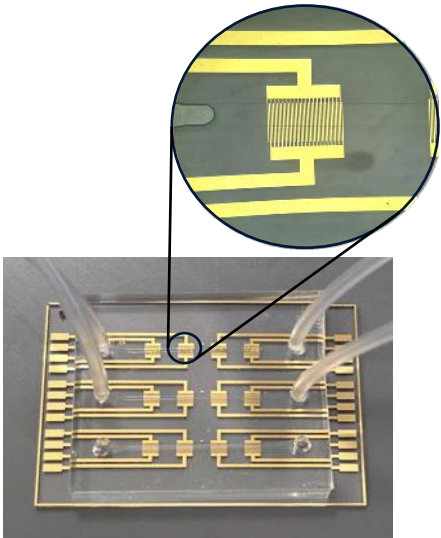
- Oxygen
- pH
- Confluence

## MVP Integration

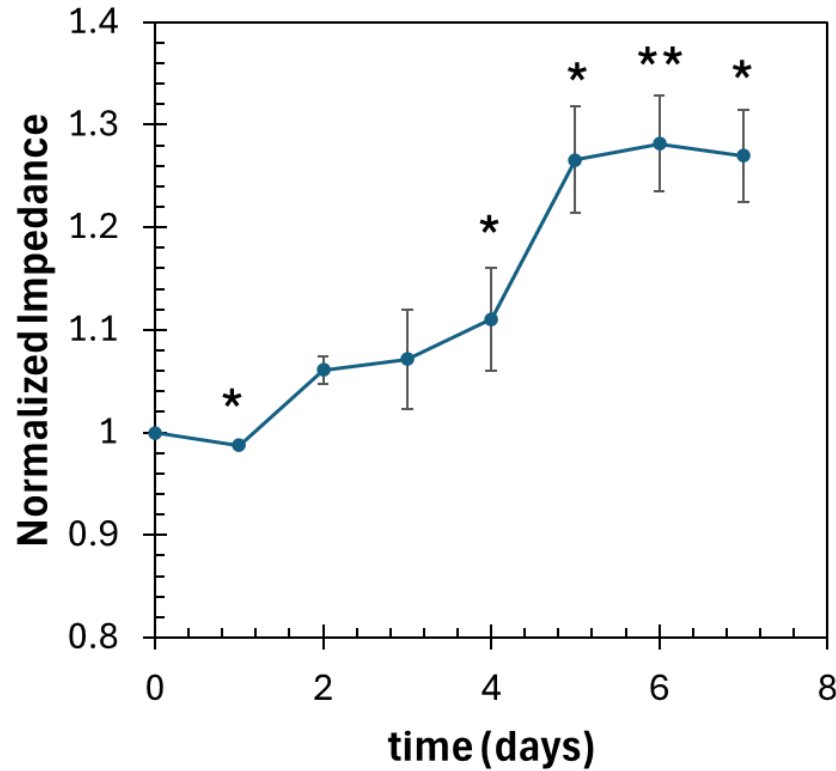
- Pump optimization
- Sensor location
- Bubble mitigation
- Media infusion and collection



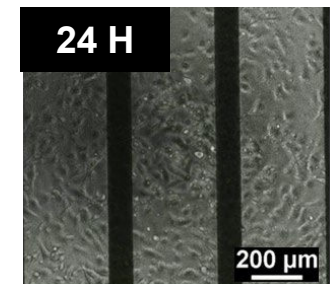
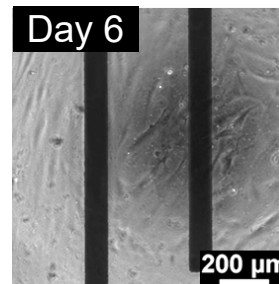
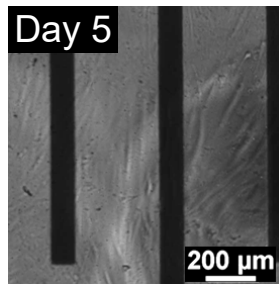
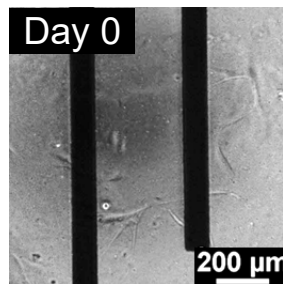
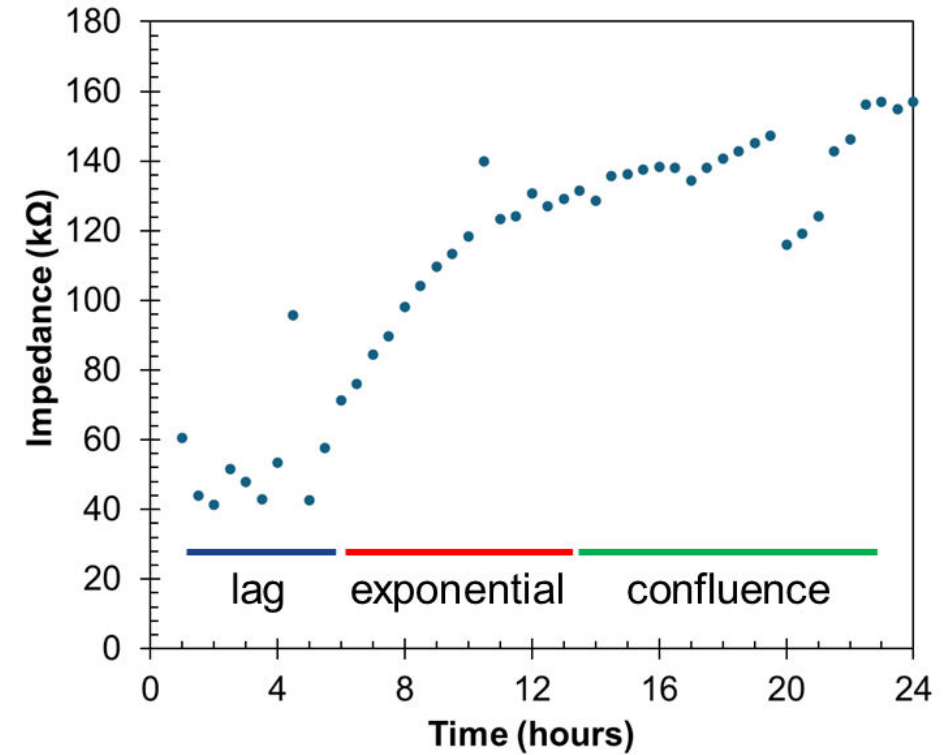
## Electrode



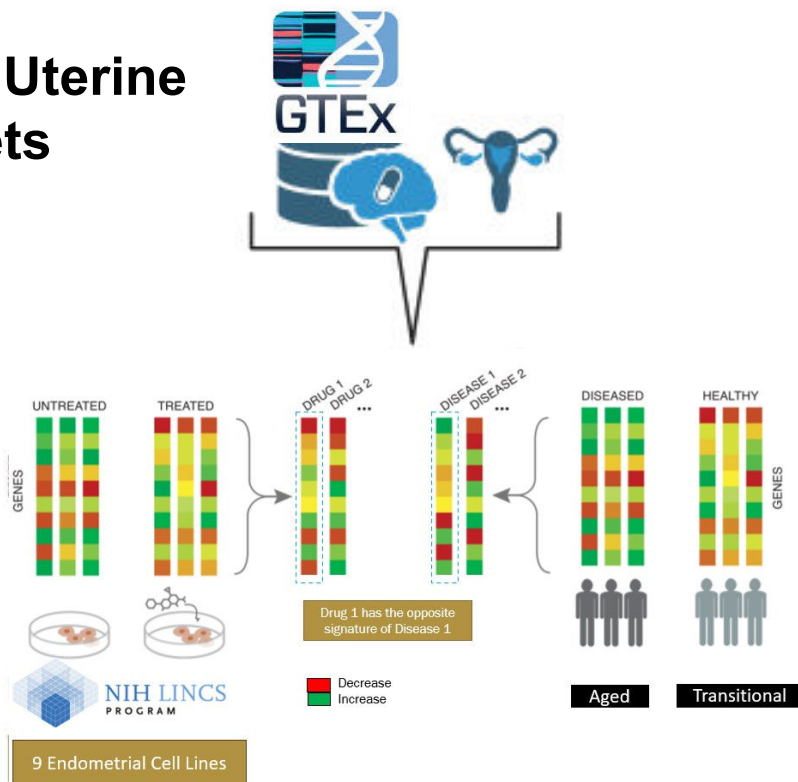
## Smooth Muscle Cells



## Epithelial Cells



## Public Uterine Datasets

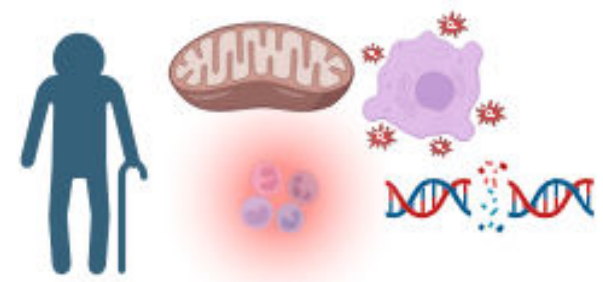
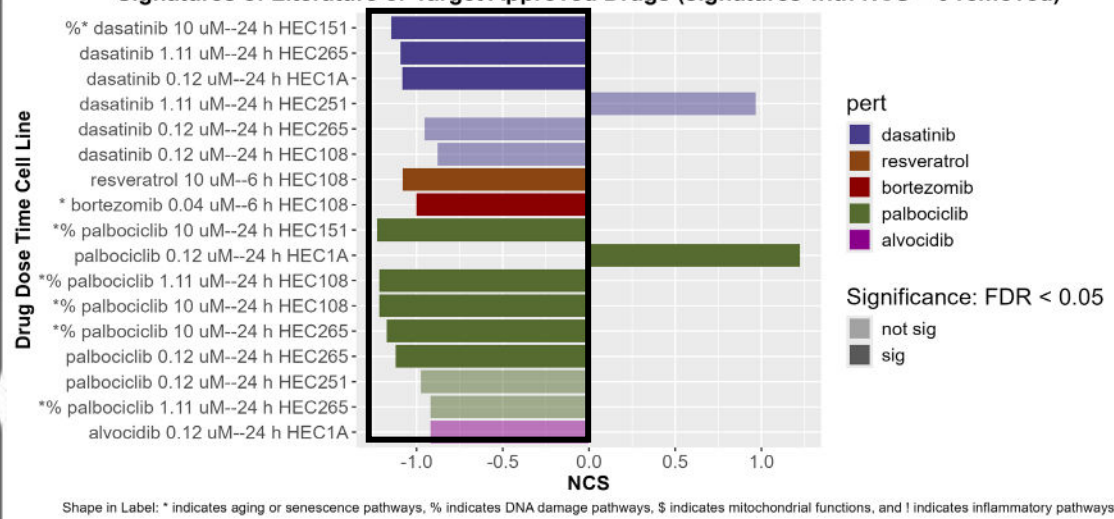


## Signature Reversion (Endometrial cell lines)



## Drug Candidate Identification

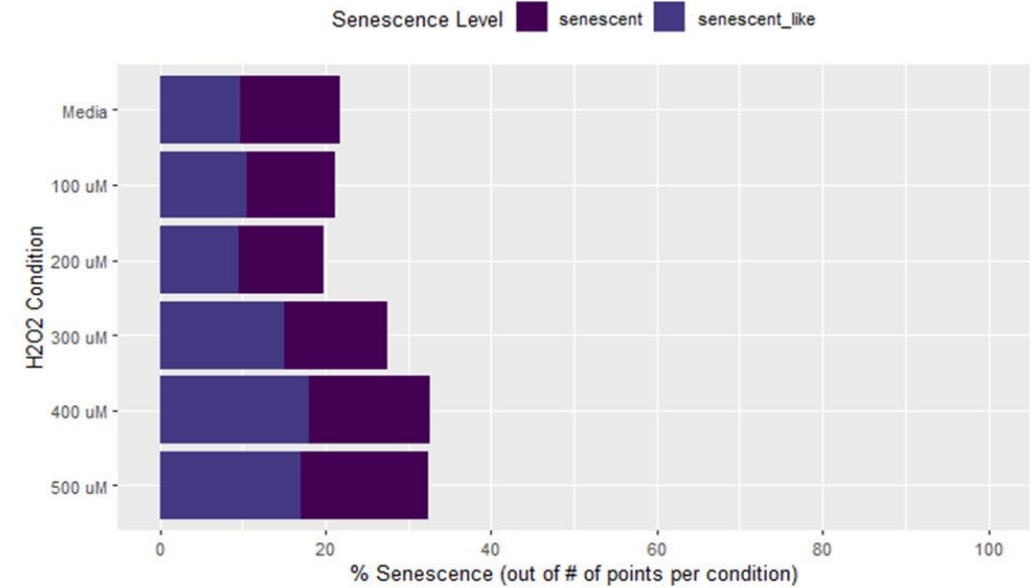
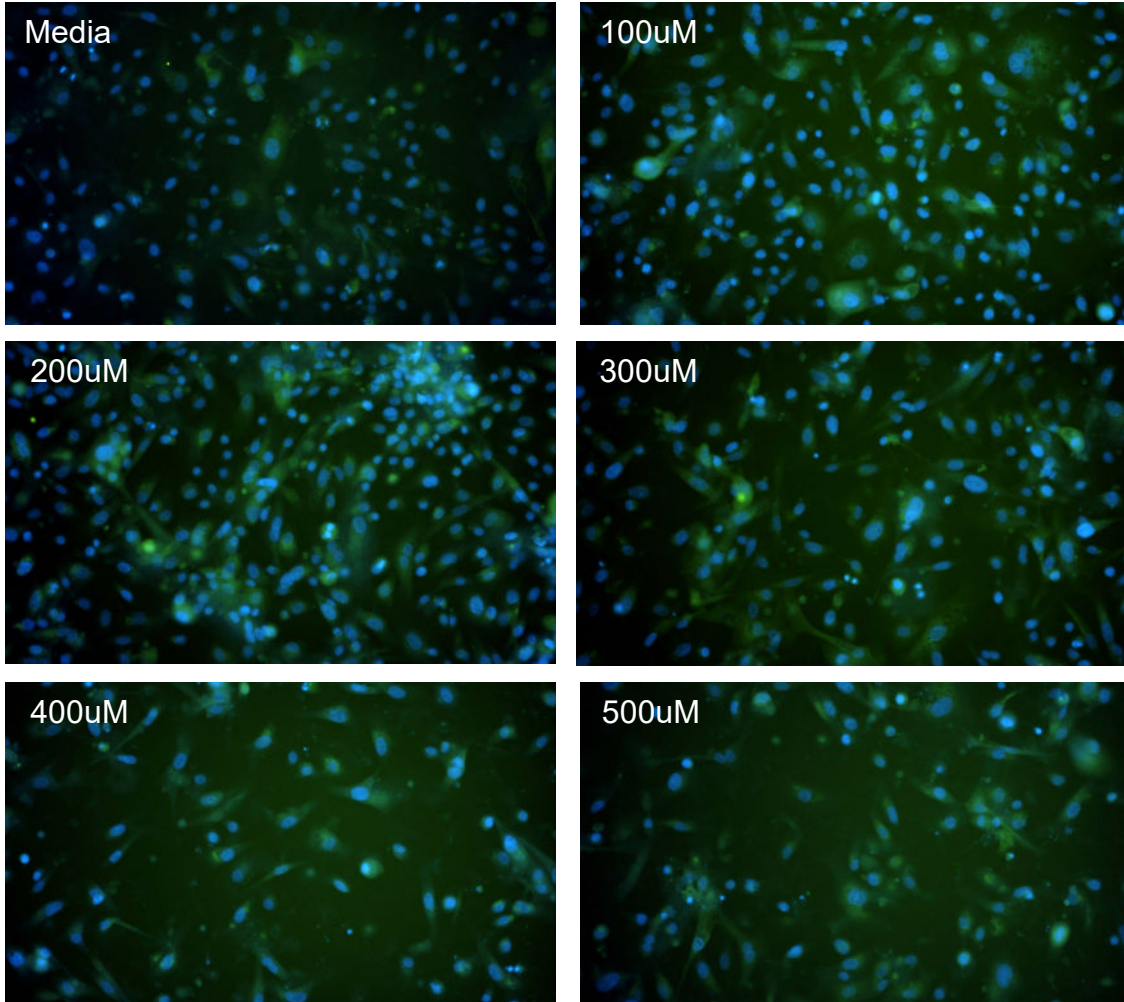
Signatures of Literature or Target Approved Drugs (signatures with NCS = 0 removed)



Drug	Supporting Evidence for Use as Drug Repurposing Candidate
<p><b><u>Bortezomib</u></b></p>	<ul style="list-style-type: none"> <li>• Protective on the uterus of ovariectomized rats (PMID: 30074329)</li> <li>• Bortezomib Prevents Acute Doxorubicin Ovarian Insult and Follicle Demise, Improving the Fertility Window and Pup Birth Weight in Mice (PMID: 25251158)</li> <li>• Drug target in aging/senescence pathways</li> <li>• Significant negative NCS score with signature reversion</li> <li>• Perturbation signature with aging/senescence pathways enrichment</li> <li>• Perturbation signature with other aging hallmarks enrichment</li> <li>• Increase in ESR2 &amp; PGR gene expression</li> </ul>
<p><b><u>Palbociclib</u></b></p>	<ul style="list-style-type: none"> <li>• Studies for endometrial cancer</li> <li>• Drug target in aging/senescence pathways</li> <li>• Significant negative NCS score with signature reversion</li> <li>• Perturbation signature with aging/senescence pathways enrichment</li> <li>• Perturbation signature with other aging hallmarks enrichment</li> <li>• Increase in IGFBP1 gene expression</li> </ul>

\*Note certain pathways/gene are enriched/expressed in specific endometrium cell lines, doses, or time-points

# Drug Repurposing – Assays and Characterization



Measurement	Young	Aged
Estrogen and Progesterone Receptor Expression	Young > Aged	
IGFBP1 Expression <sup>27,39</sup>	Young > Aged (10.3 vs 15.41 ng/mL)	
Beta-galactosidase Expression	Young < Aged	
DNA Damage <sup>31</sup>	Young < Aged	
Smooth Muscle Contractility <sup>36</sup>	Young > Aged (6 mN vs 2 mN)	
Endometrial Thickness <sup>40</sup>	Young > Aged (6 mm vs 4 mm in luteal phase)	
RNA-Seq (sample number & age)	n=36 (< 40) <sup>41</sup> , n=12 (< 30) <sup>42</sup>	n=106 (>=40), n=12 (>45) <sup>42</sup>
miRNA (sample number & age) <sup>43</sup>	n= 3 (< 35), n=12(<30) <sup>42</sup>	n=27 (>35), n=12 (>45)

## Future Plans

- Extended culture (>35days)
- Characterization of aging
- Drug exposure



- NIH NCATS TCIS 2.0 -1UG3TR005857-01

- Carrie German, Ketan Bhatt, Kevin Roehm, Dustin Haithcock, Katie Dietz

- Dr. Matthew Anderson, Dr. Begum Mathyk

- Juan Gnecco

- Shuo Xiao

- Aaron Rogers, Rich Bolinger, Grant Vellinger

- Gwen Fewell, Jenna Rosano

