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# **Functional In Vitro Systems for Drug Discovery**

**Michael L. Shuler**

Biomedical Engineering

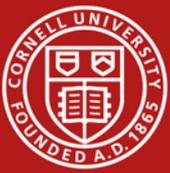
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# ***In vitro* Replacements for Animals and Humans Are Needed in Drug Development**

- Animal studies are expensive, long, and not particularly predictive of human response
- Currently only 1 in 10 drugs entering human clinical trials emerge as FDA approved products

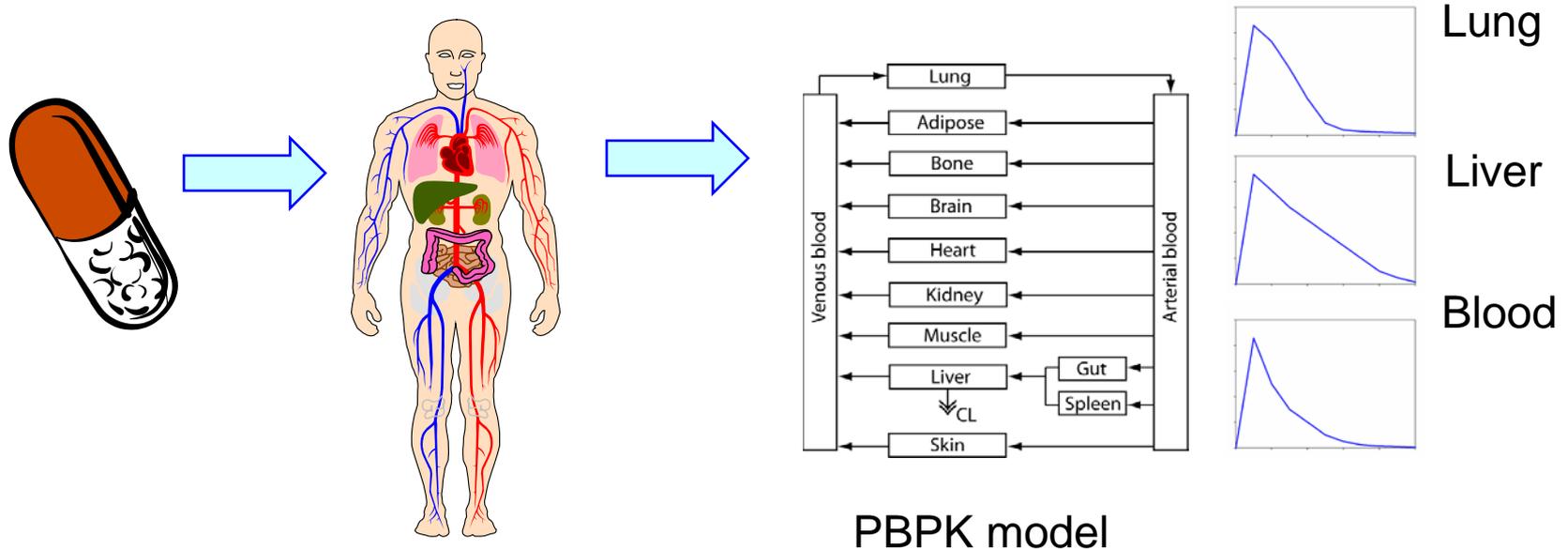


# Alternatives

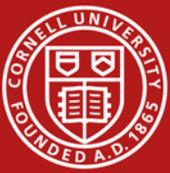
- Can we reduce use of animals and make the remaining animal studies more useful?
- Can we make a realistic human surrogate?
- Can we integrate advances in understanding of toxicity at the molecular level with predictions of dose effects in humans?



# Mathematical Models Can Predict Drug Distribution



- PBPK (Physiologically-Based Pharmacokinetic) computer models treat human body as a series of interconnected compartments
- Compartments are reactors, absorbers, or surge tanks
- PD (pharmacodynamic model) predicts pharmacological effect



# Combine Cell Cultures and PBPK Models?

- Important biological mechanisms may be missing in PBPK-PD models; can reliability of predictions be improved?
- Can a realistic, physical in vitro model of animals or humans be constructed based on a PBPK?
- Can we substitute living cells or tissue engineered constructs for differential equations?



# Cell Culture Analog (CCA)

- Physical Representation of PBPK
- Idea in 1989
- First Publication:  
Sweeney, et al. (1995). *Toxicology In Vitro*; 9:307-316.
- Macroscopic system testing mechanism of toxicity of naphthalene in rodents and why responses differ in mice and rats
- US Patent # 5,612,188

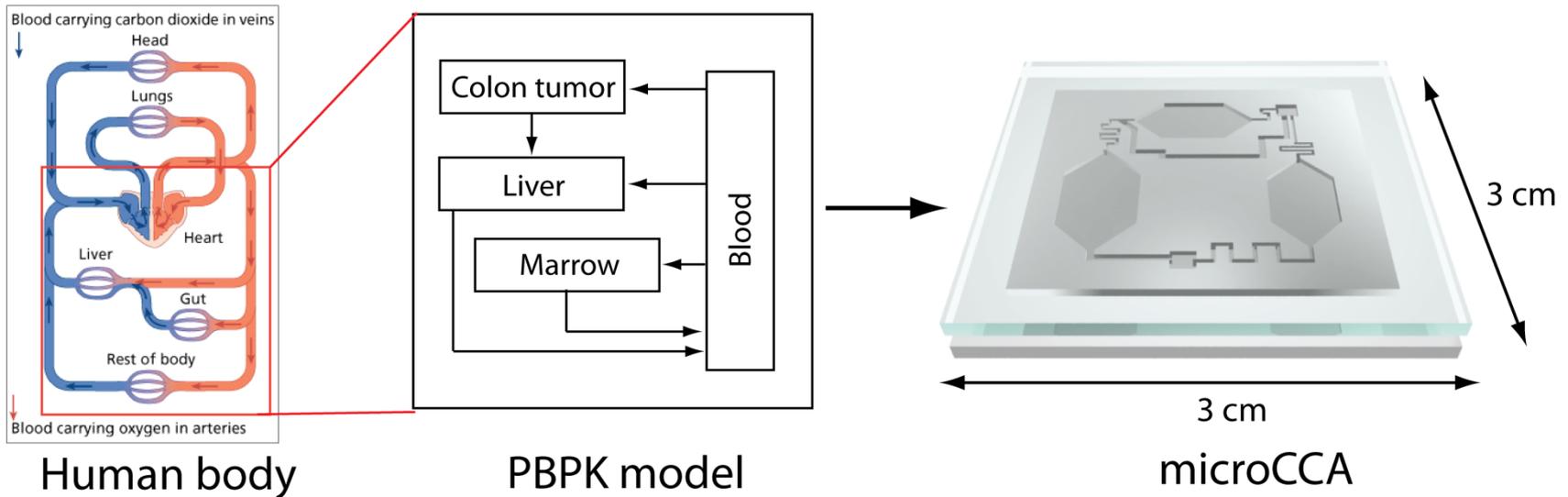


# Macro CCA

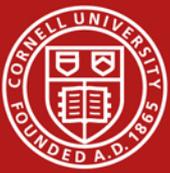
- Relatively inefficient and hence costly
- Microscale would allow many more tests with mass produced devices using minimal cell material decreasing cost per result by several orders of magnitude
- Since microscale natural length scale in body, easier to recapitulate physiological response



# A Micro Cell Culture Analog is the Physical Realization of a PBPK/PD Model



“Body-on-a-Chip”, (Newsweek, October 20, 2005)



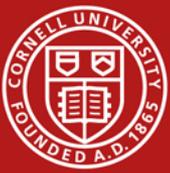
# Micro CCA

- Work initiated in 1998 (with Greg Baxter)
- US Patent # 7,228,405
- Recognized in Newsweek in their “Big Ideas” issue



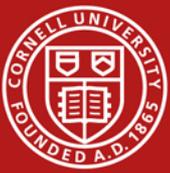
# What Are The Advantages of a CCA?

- Realistic dose dynamics
- PBPK-CCA comparison provides direct test of plausibility of mechanism
- Assist in extrapolation of animal to human predictions



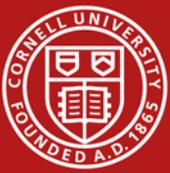
# Living Systems on a Chip

- Nature Technology Feature;  
Nature 471:661, (2011).
- Overview of related technology including examples of both organs-on-a-chip and body-on-a-chip models
- Also see Sung & Shuler, Bioprocess. Biosyst. Eng. 33:5 (2010).  
For review of related technology
- HuRel has licensed Cornell patents and is commercializing basic concept. (Shuler on SAB)



# What Are the Challenges in Making a Micro CCA?

- Long term (96 h) fluid recirculation
- In situ data analysis
- Common blood surrogate
- Biggest barrier is authentic biological constructs – recapitulate fully biological function

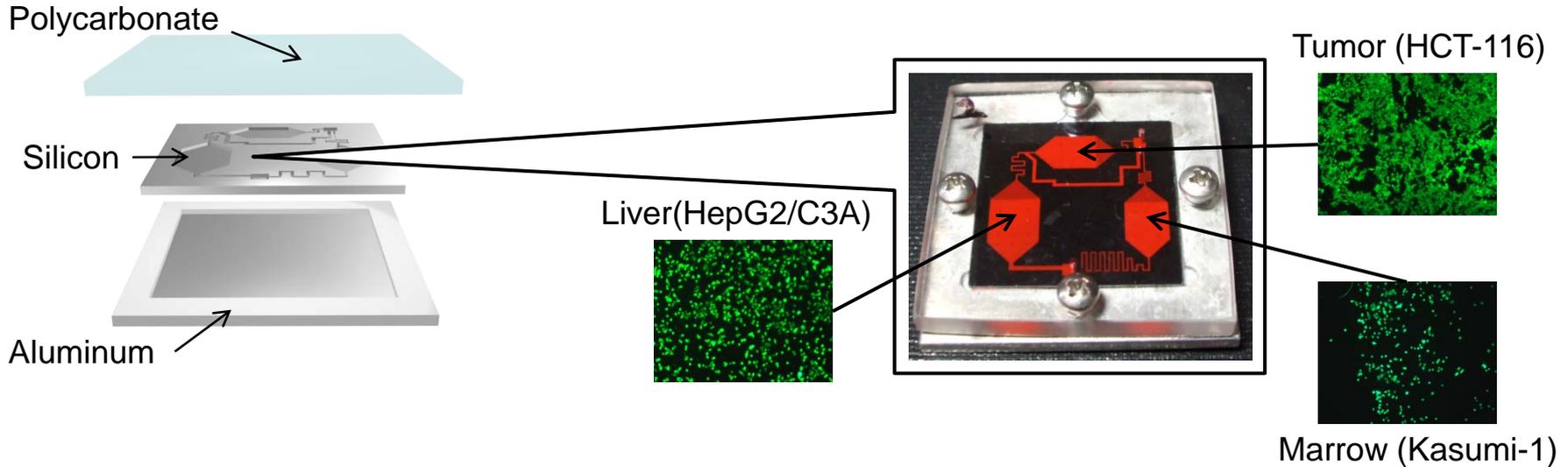


# Ideal Attributes for Biological Constructs Include:

- Mimic biological response of tissues
- Multiple cell types and functional (e.g. electrically active)
- Under 200  $\mu\text{m}$  in depth is desirable
- Reproducible
- Store and then use (cryopreserve)



# Design and Assembly

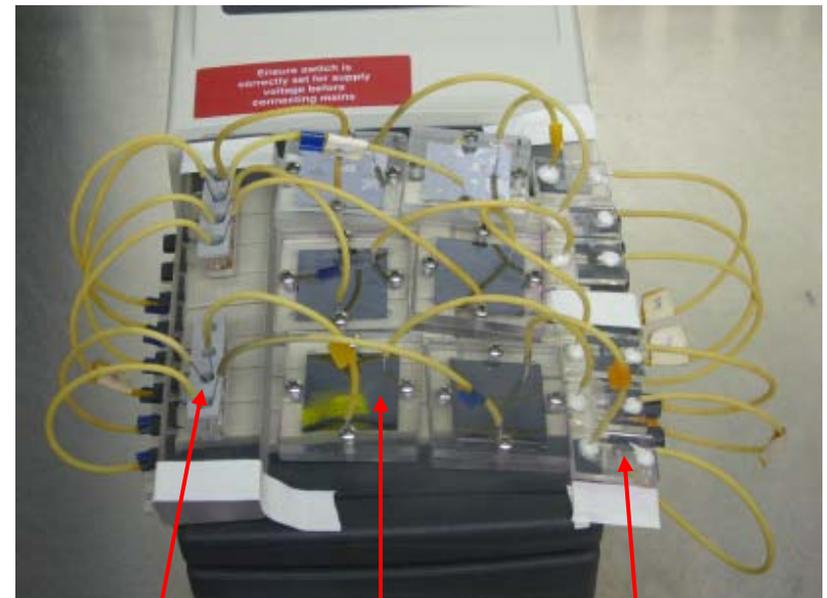
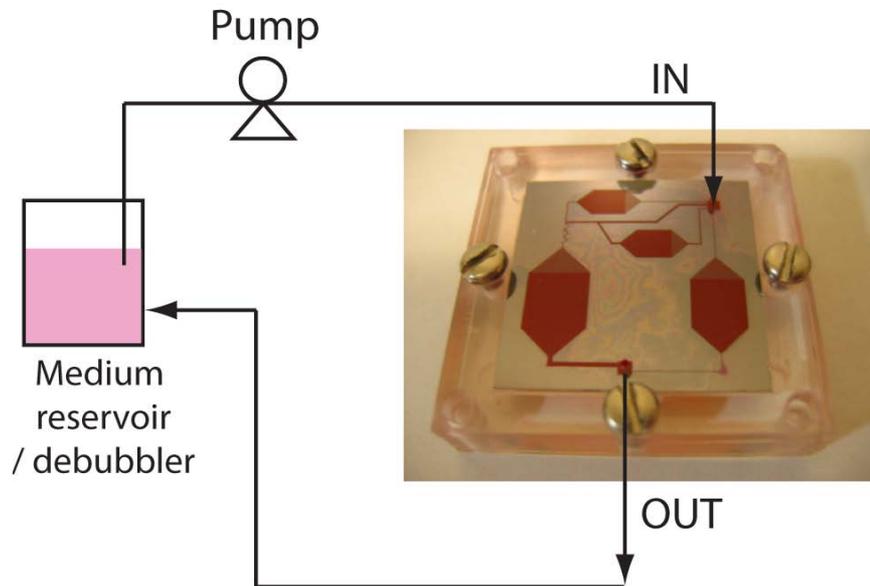


	Tumor	Liver	Marrow
<i>Calculation</i>			
Residence time (s)	70	52	128
Velocity (um/s)	71.5	160.7	53.6
<i>Measurement</i>			
Residence time (s)	69.7±3.8	49.7±3.5	136.5±2.9
Velocity (um/s)	68.9±1.6	167.9±6.2	58.6±0.5

- Fabricated from silicon
- Based on previous  $\mu$ CCA
- Designed to test drugs for colon cancer
- Liver/tumor/marrow
- Flow residence times are matched to physiological values



# System Operation



Medium reservoir

μCCA

Bubble trap

Medium is recirculated ( $200\mu\text{L}$ ) to mimic the body's recirculation 6~8 chips, operating time: 3 days



# First Practical $\mu$ CCA System

- Initial Publications
  - Sin, et al., 2004, Biotechnol. Prog. 20:338.
  - Viravaidya, et al., 2004, Biotechnol. Prog. 20:316.
  - Viravaidya, et al., 2004, Biotechnol. Prog. 20:590.
- “Liver”, “lung”, “fat”, and other tissues
- Integration of PBPK and experimental device critical to interpretation
- Demonstrates naphthaquinone rather than naphthalene epoxide is generated in liver and causes lung cell death in mice
- PBPK explains why mechanisms of death in mice (lung) differ from rats; timing of naphthaquinone release and resynthesis of glutathione.



# PBPK Guided Device Important

- Multicompartment system by itself not enough to get realistic prediction
- Need compartments to communicate in realistic, time dependent manner
- To build totally realistic system difficult, but PBPK of device can yield fundamental parameters which allows extrapolation to whole human or animal response



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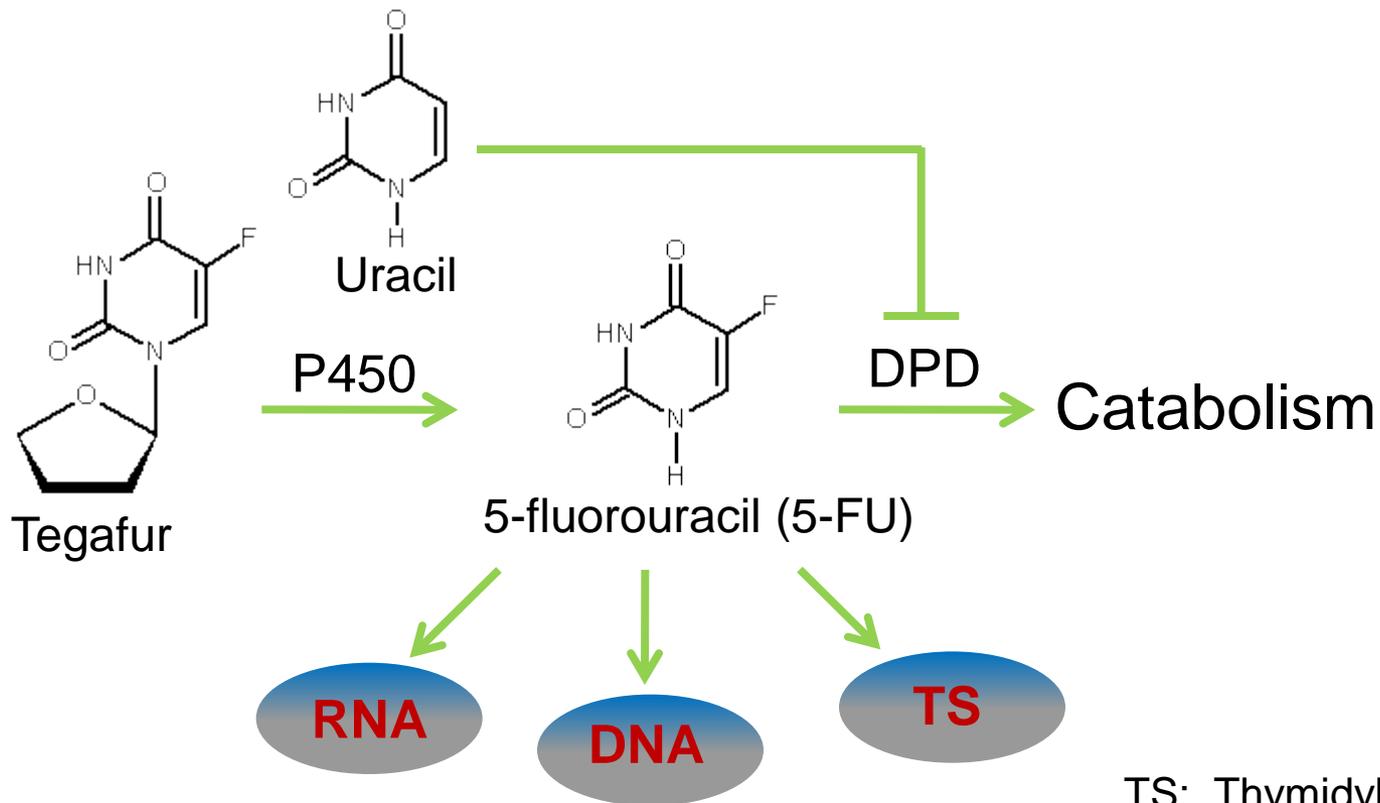
# Can the “Body-on-a-Chip” Approach Be Used With Other Combination Treatments for Cancer?

Sung & Shuler. 2009. Lab-on-a-Chip,  
9:1385-1394.



# Colon Cancer & UFT

- Third-leading cause of cancer-related death in USA
- UFT: Uracil (modulator) + Tegafur (prodrug of 5-FU)



TS: Thymidylate synthase  
DPD – Dihydropyrimidine dehydrogenase



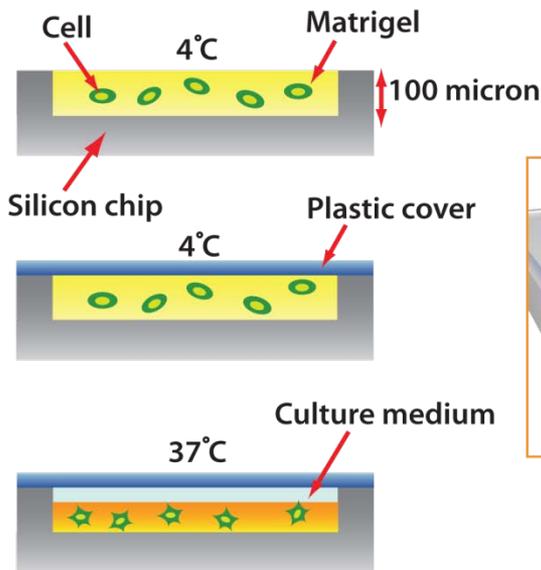
# Minimal Cell Types Required

- Liver – Metabolism – HepG2/C3A
- Colon Cancer – Target – HCT-116
- Marrow (Myeloblast) – Dose-limiting cell type (Kasumi-1; a suspension cell)



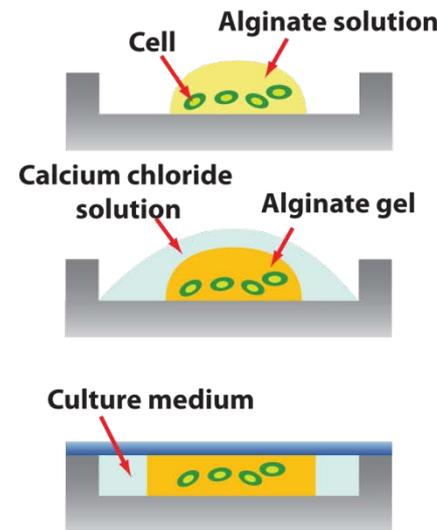
# Hydrogel Cell Culture

3-D cell culture can elicit more authentic cell behavior



## Matrigel

- Protein mixture secreted by mouse tumor cells
  - Thermal curing (liquid at 4°C, gel at 37°C)
- Shrinkage during gel formation provides space for medium perfusion

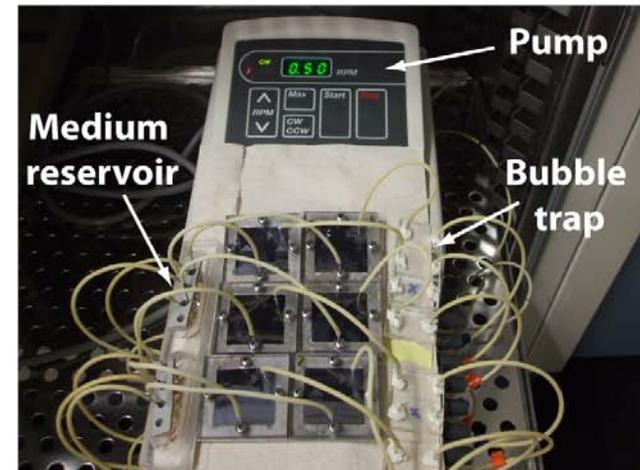
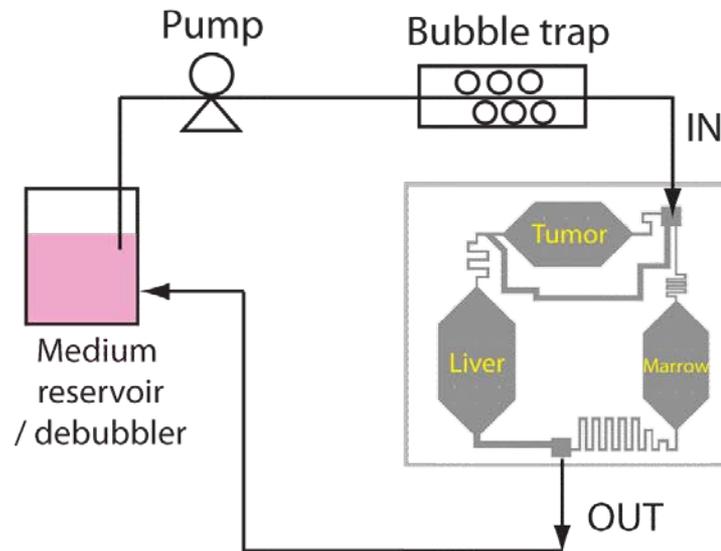


## Alginate

- Biopolymer extracted from seaweed
- Gel formation by calcium chloride ions
  - Medium flows around gel



# System Operation

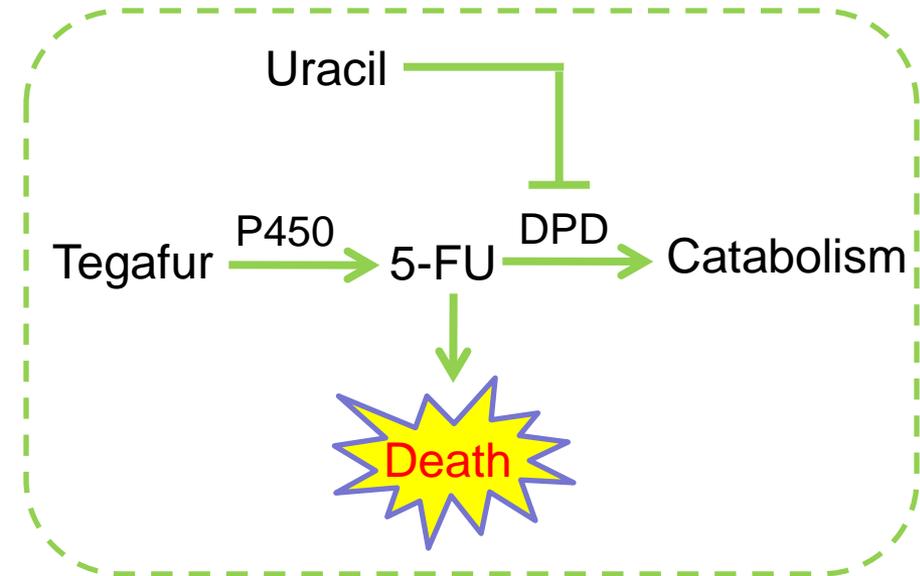
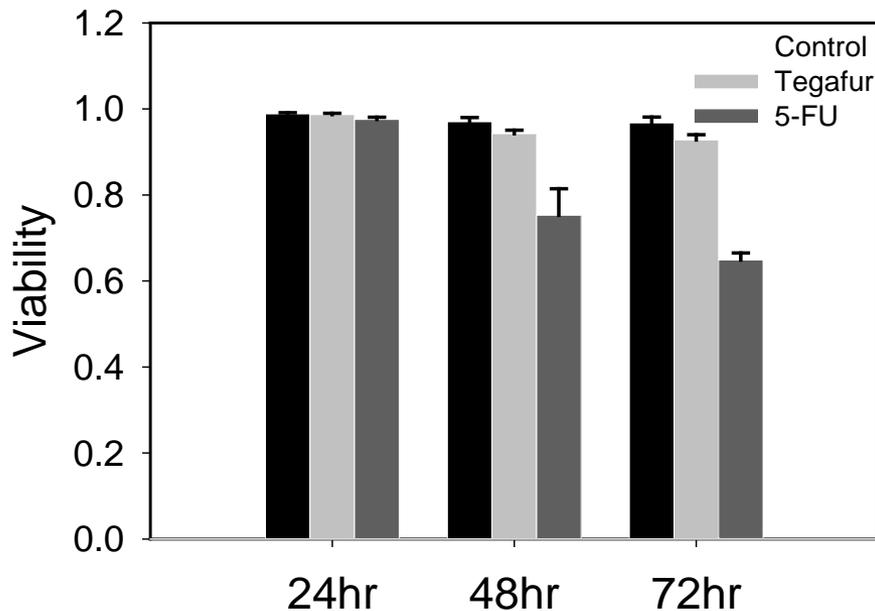


- Medium in reservoir is recirculated
- 6~8 chips are run simultaneously
- Devices are operated up to 4 days

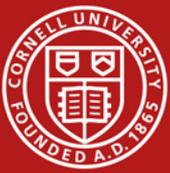


# Tegafur Toxicity (96-well)

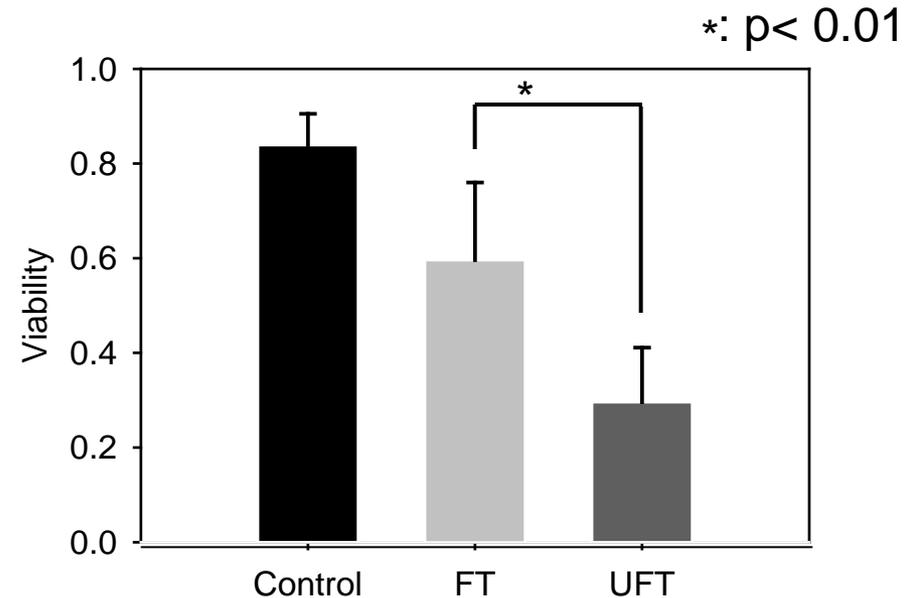
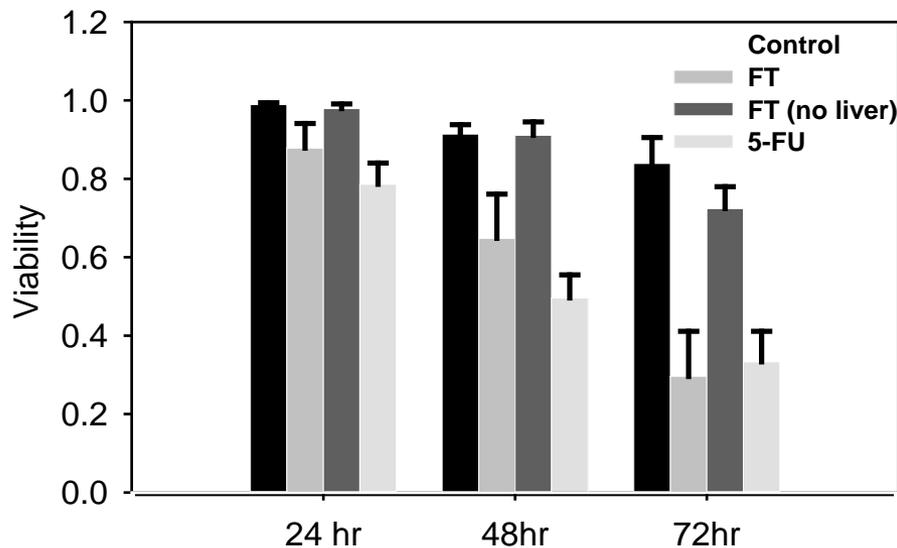
HCT-116 (colon cancer cell line) treated with 5-FU or Tegafur



- 5-FU is toxic to tumor cells
- Without metabolizing enzyme (tumor cell alone), Tegafur is not toxic



# Tegafur Toxicity ( $\mu$ CCA)

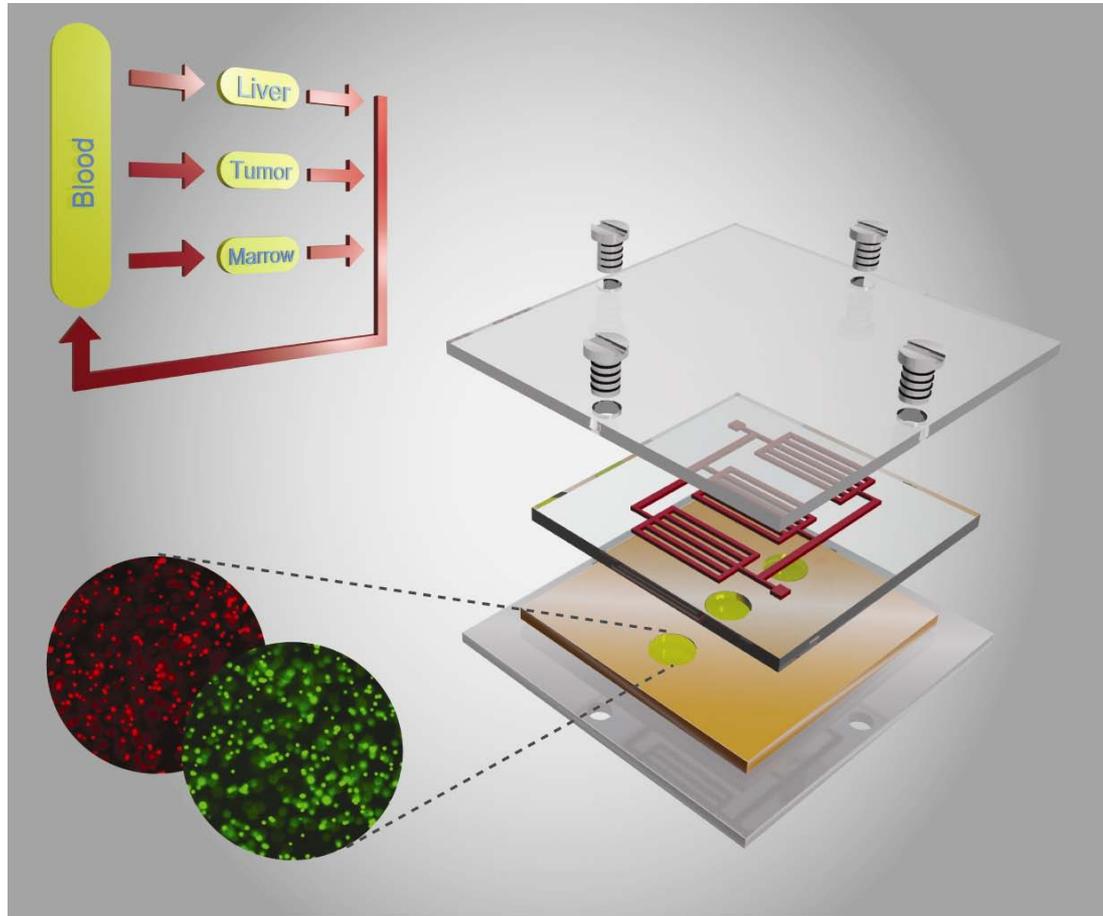


- Both 5-FU and Tegafur was toxic to tumor cells in  $\mu$ CCA
  - Liver cells were responsible for Tegafur toxicity
    - Uracil enhanced Tegafur toxicity

UFT: Uracil + Tegafur (FT)



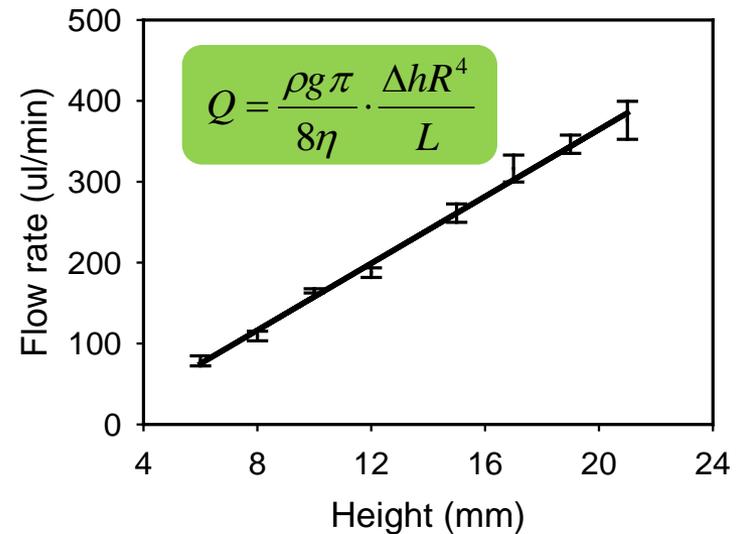
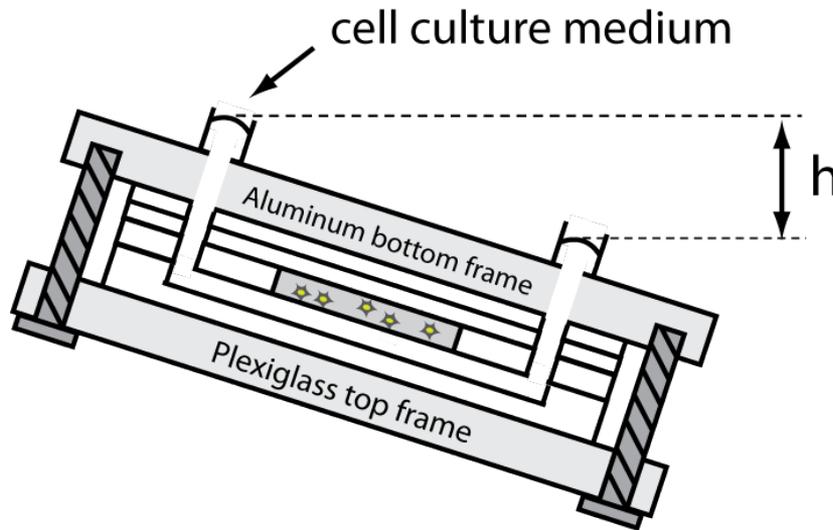
# Design to Simplify Operation



Jong Hwan Sung, Carrie Kam, Michael L. Shuler, A microfluidic device for a pharmacokinetic-pharmacodynamic (PK-PD) model on a chip Lab on a chip, 2010, (10: 446, 2010)



# Gravity-induced flow

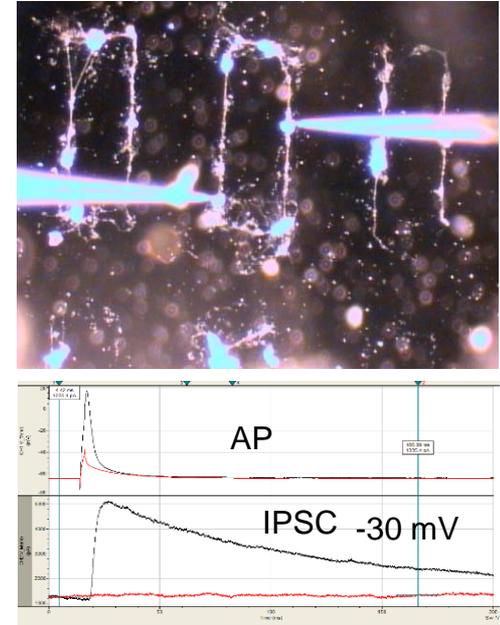


- Medium is recirculated by reciprocating tilting with rocking platform
- Gravity-induced flow naturally eliminates bubble problem
- More efficient assembly and operation of device
- Pumpless system!
- Higher Throughput
- Reduced assembly time & skill required

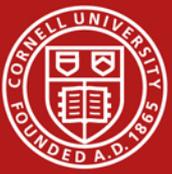


## Functional In Vitro Systems

- Functional *in vitro* systems are assays created to fill the void between single cell assays and live animals/humans in a controlled high-throughput reproducible environment.
- These sub-systems of animals or humans can include:
  - Organs (i.e. lung, heart, pancreas, etc.)  
or
  - Control systems such as: motor control, memory formation, cardiac pacemaking, etc.

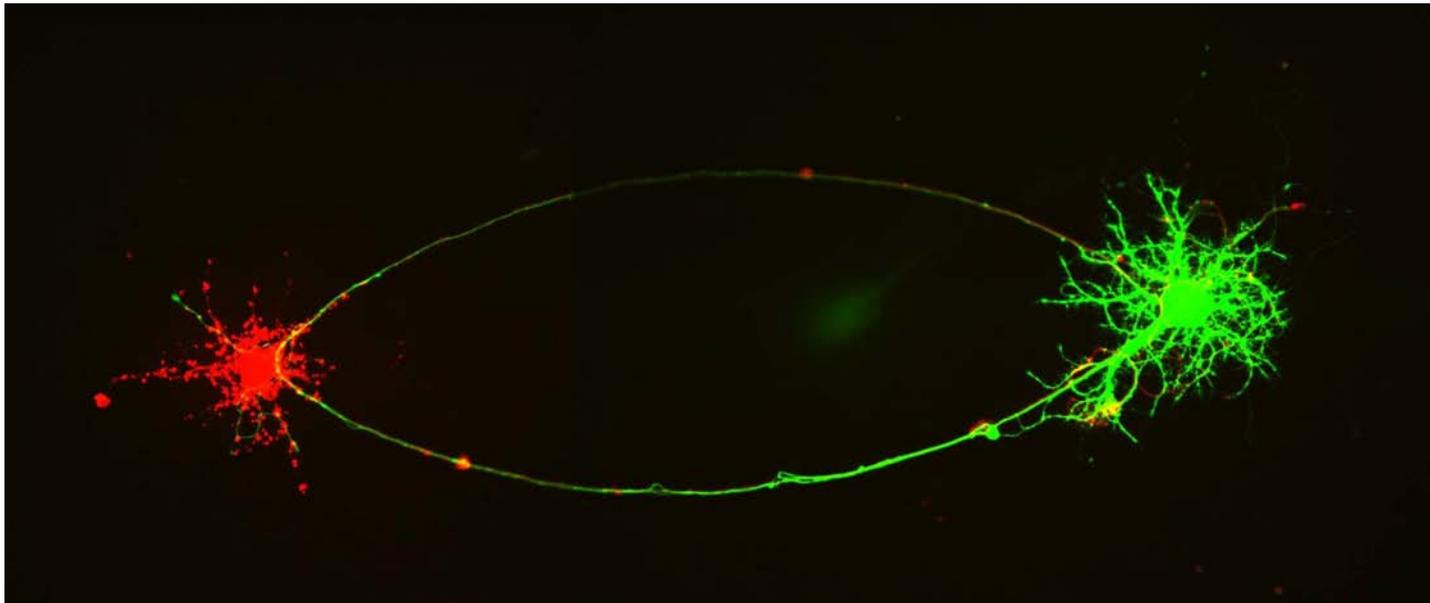
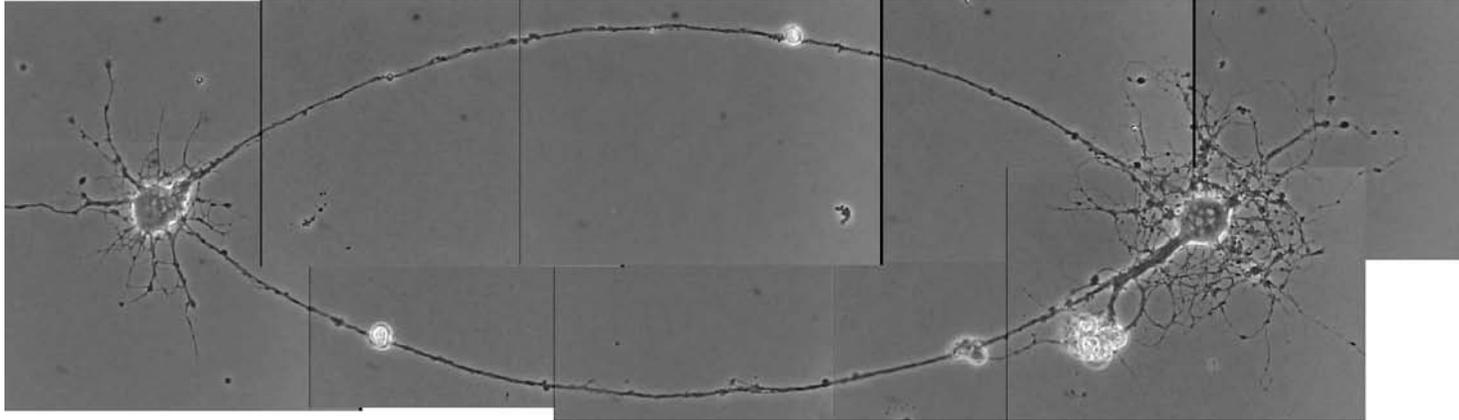


Engineered Neuronal Networks for Alzheimer's research



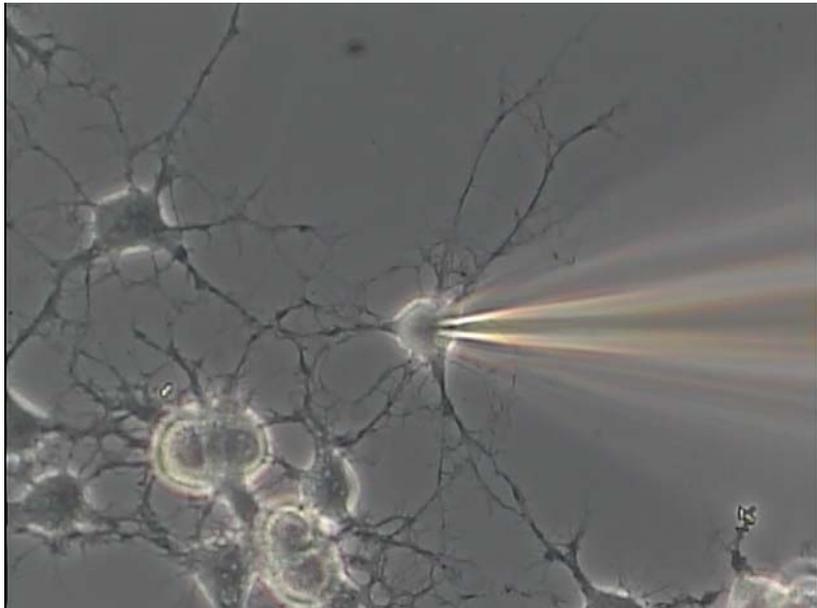
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# Advanced Hippocampal Two-Cell Networks

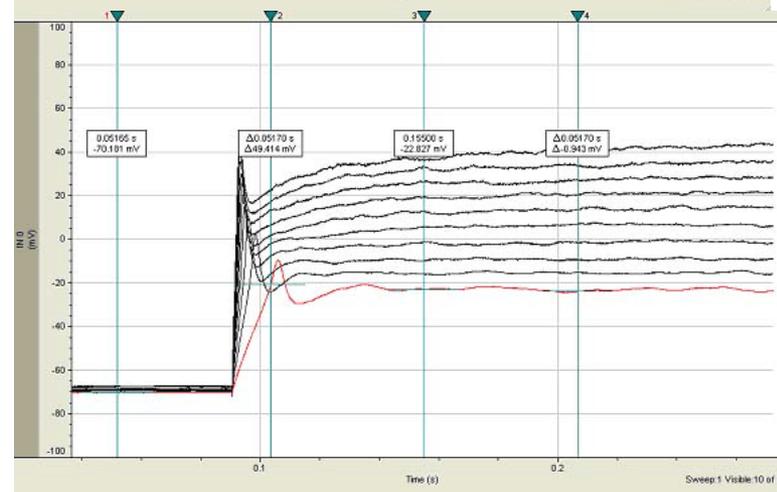
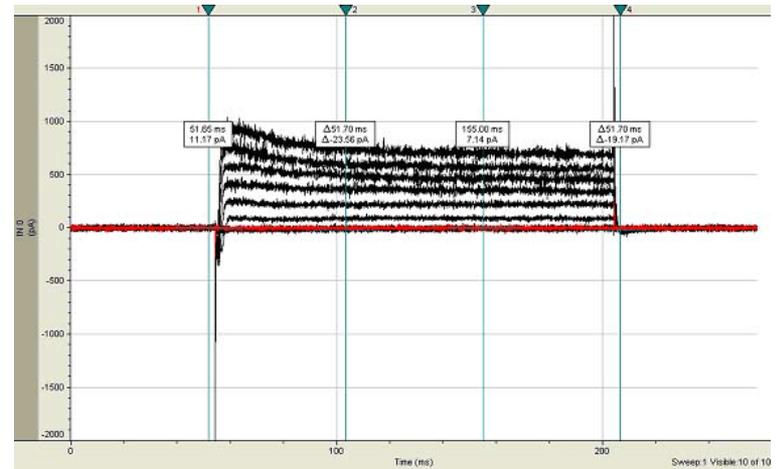


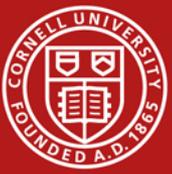


# Hippocampal neurons two months post plating, positive electrical characteristics



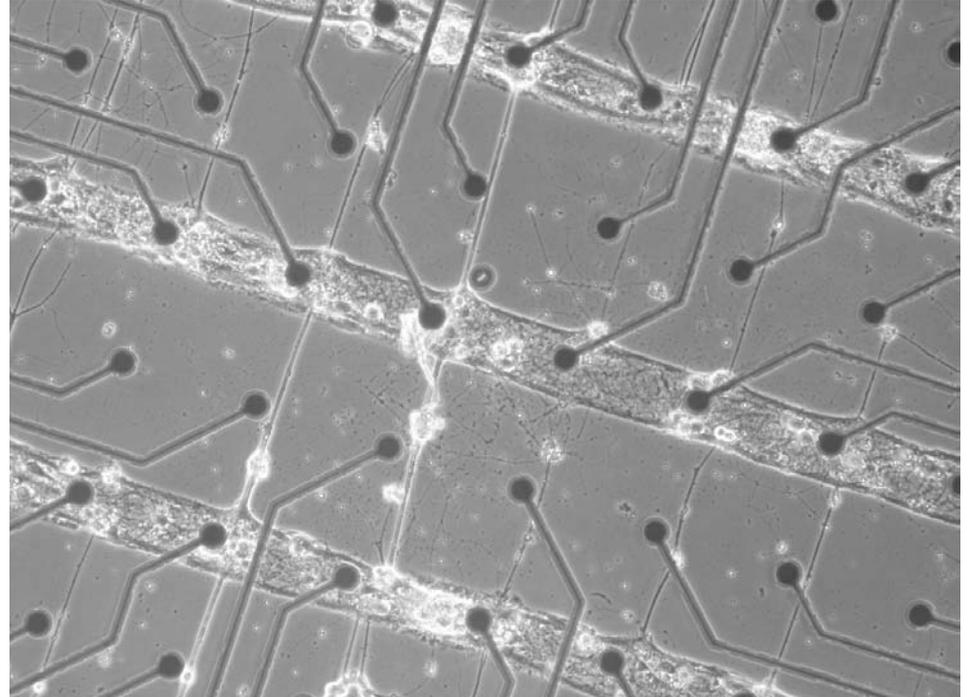
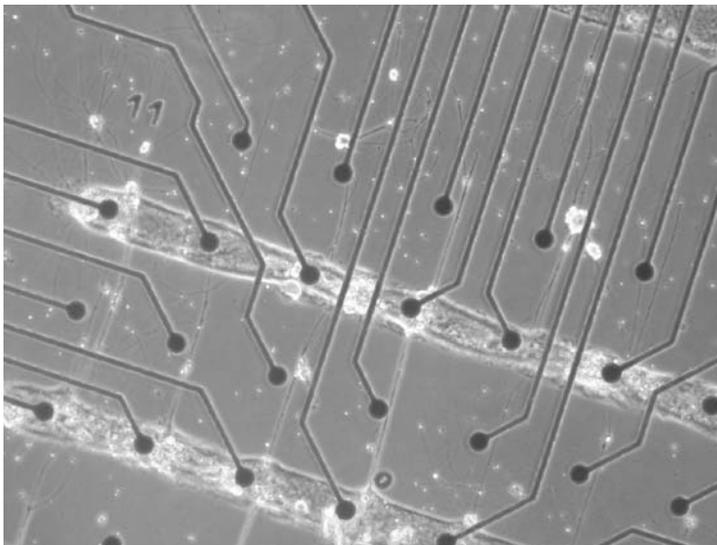
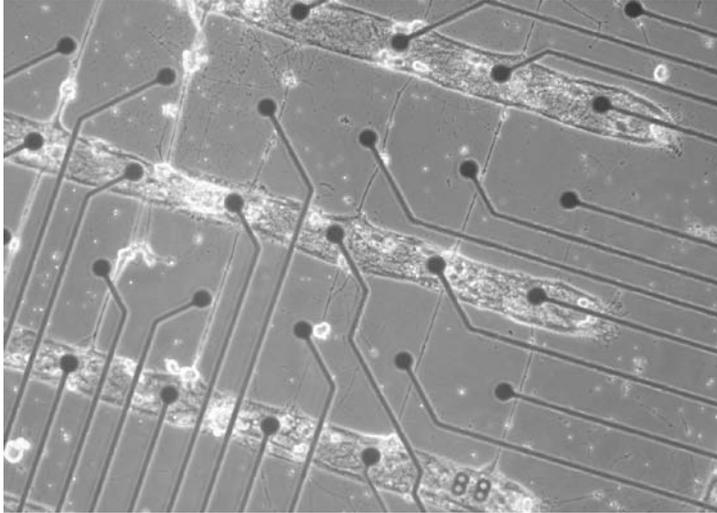
- Inward and outward currents evident in current clamp mode
- Action potential seen firing in voltage clamp mode





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# Hippocampal neurons, Day 18, NBActiV



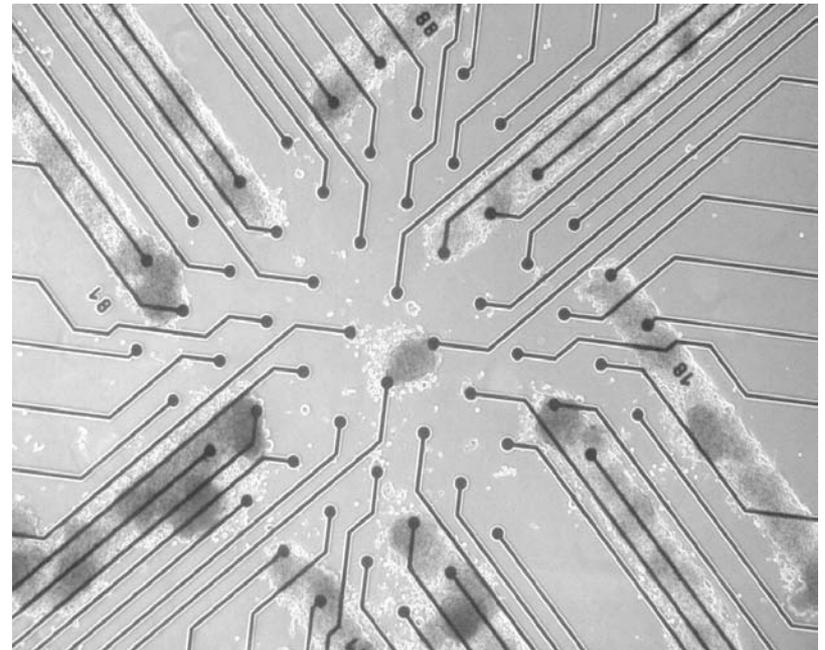
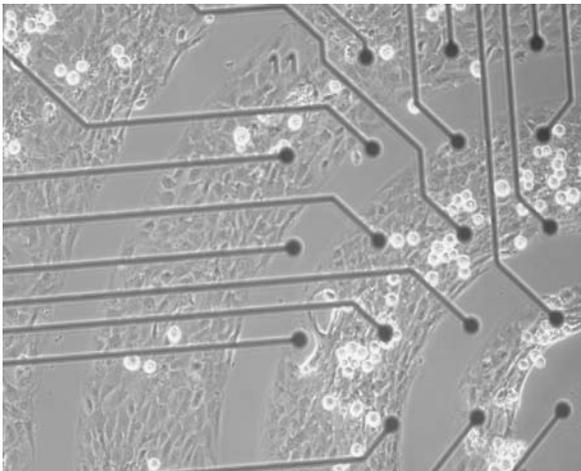
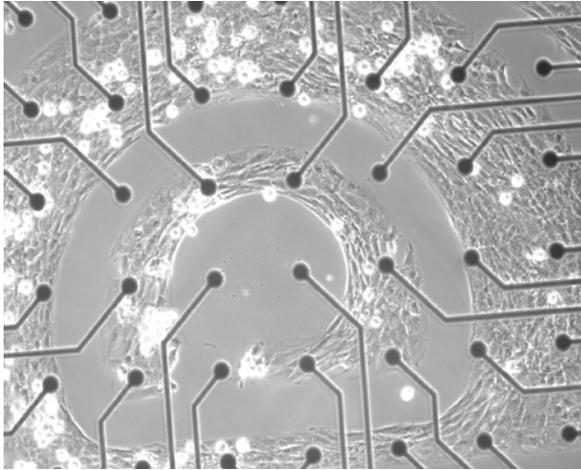
Study what neurotransmitters are involved in measured network activity.

Glutamate through AMPA receptors:  
NBQX (Block transmission)



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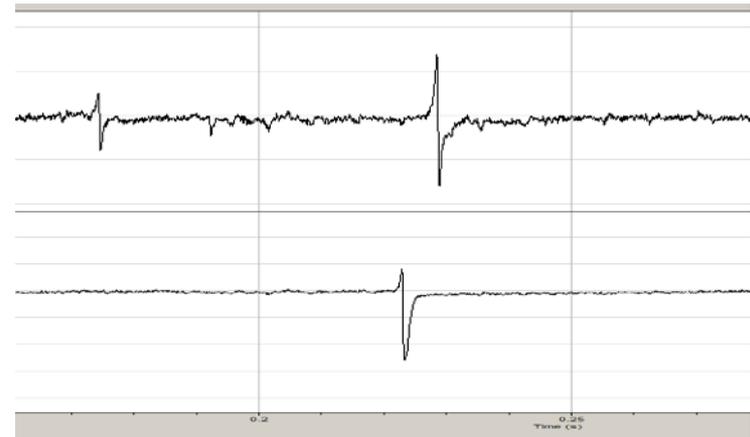
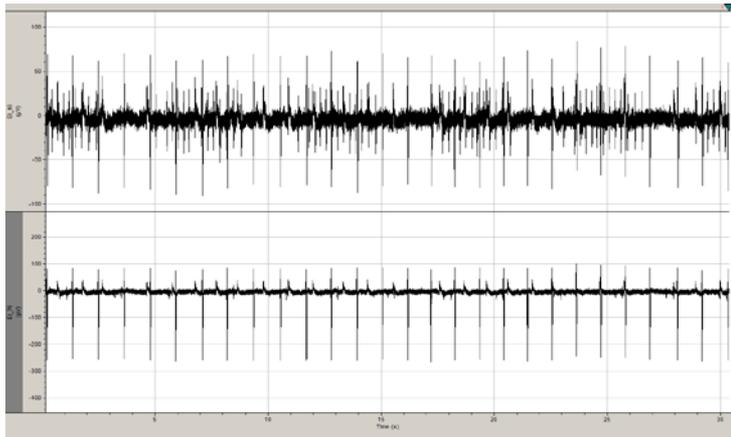
# Cardiac Patterns/Microelectrode Arrays



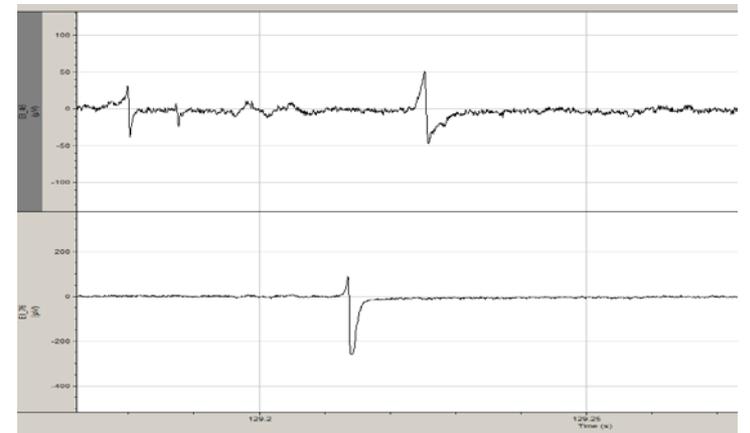
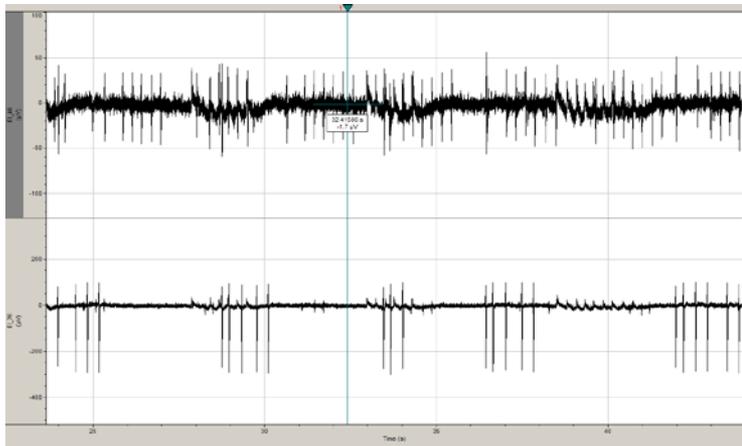


# Effect of Antibiotic Sparfloxacin (Fibrillation and QT prolongation)

Field potential recording before Sparfloxacin addition



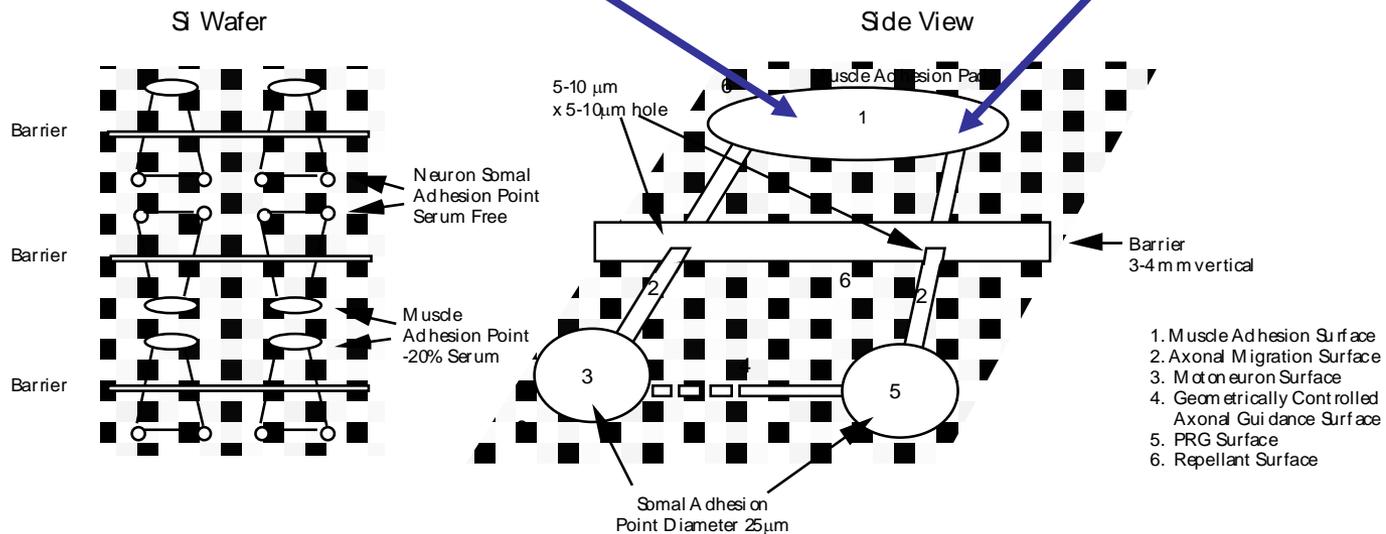
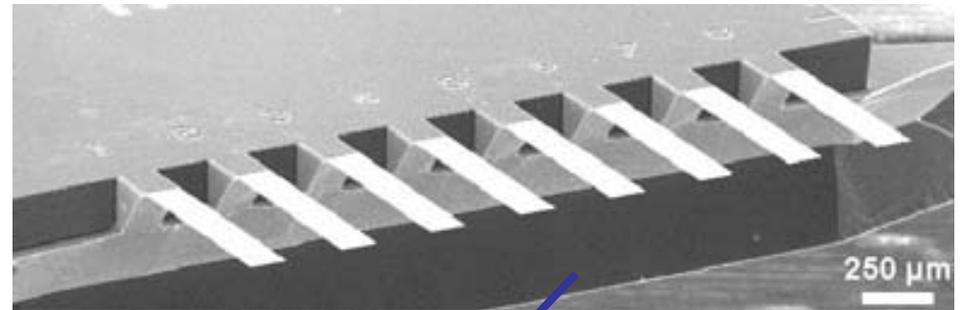
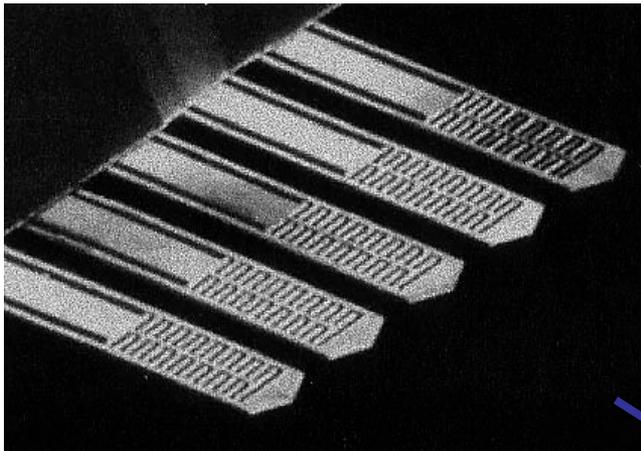
Field potential recording before Sparfloxacin addition





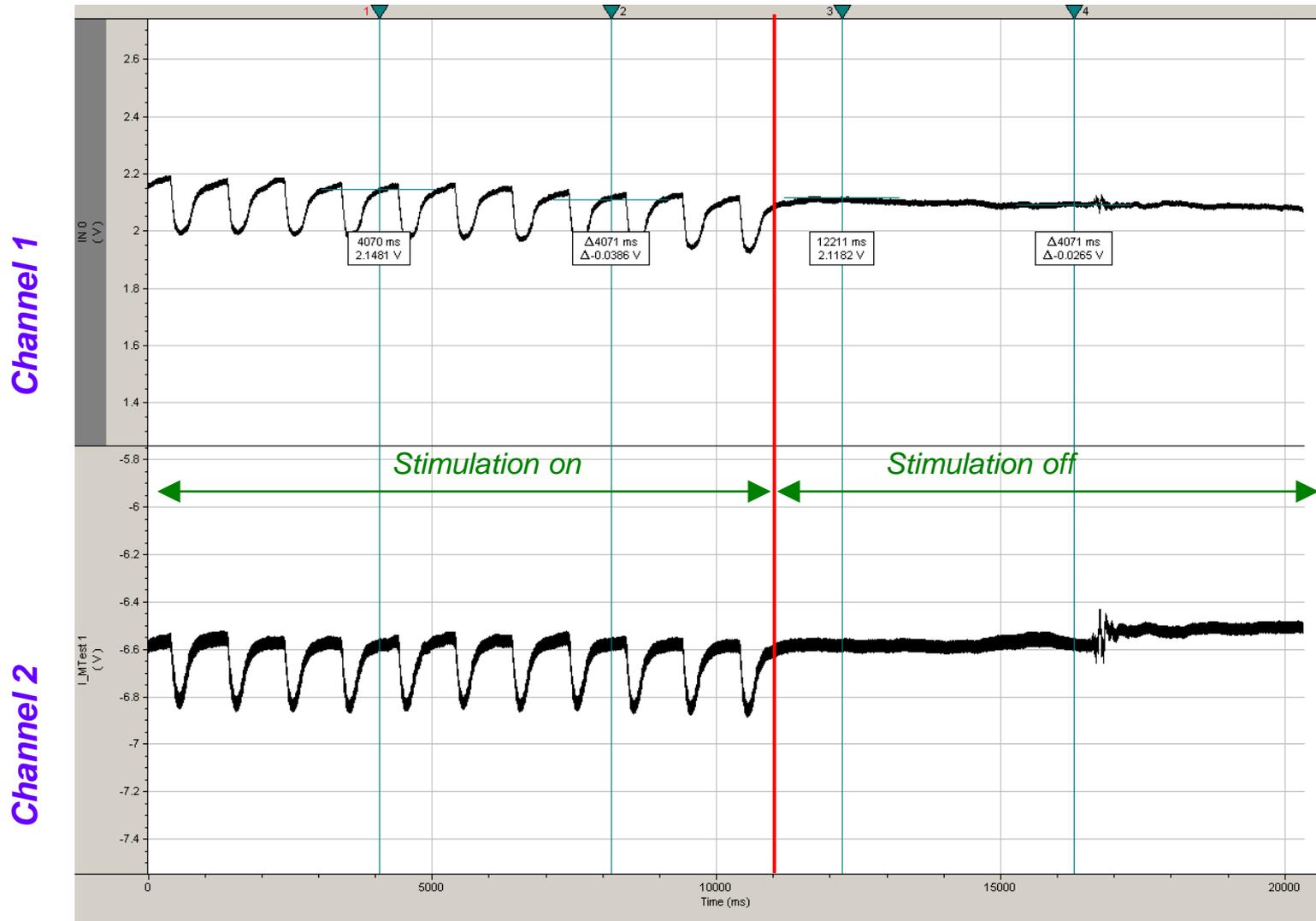
# Experimental System Design

Fabricate custom cantilever arrays to monitor muscle cells in reflex arc circuit stimulated by the motoneuron or mechanical force



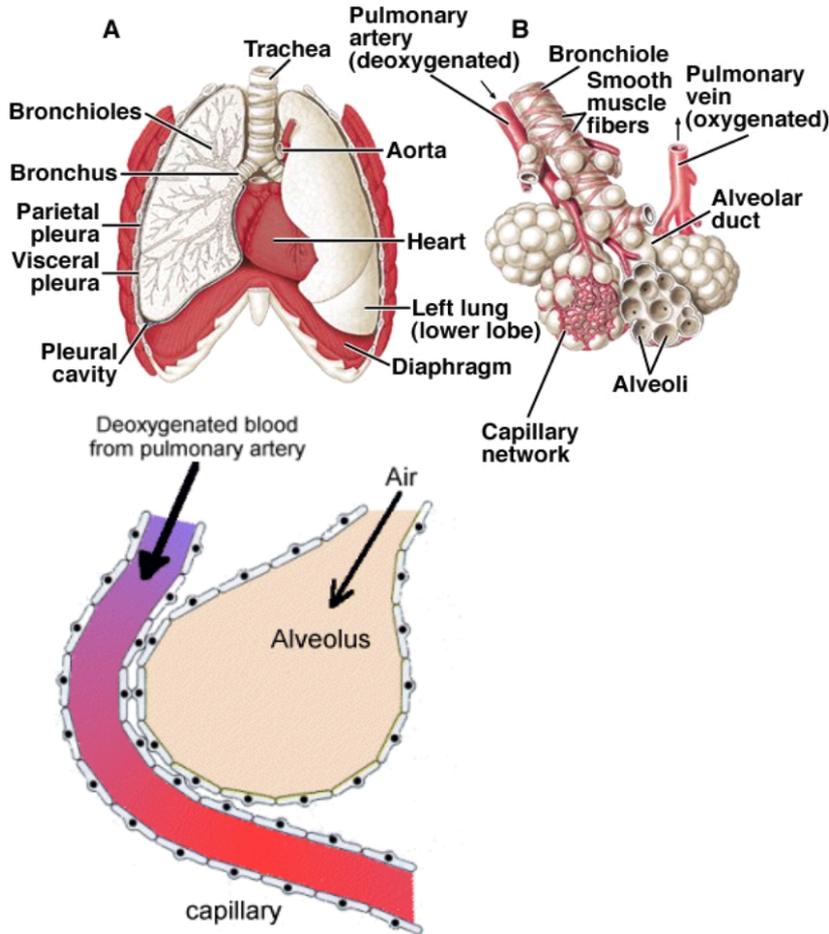


Periodic deflections due to stimulation were measure on the cantilever

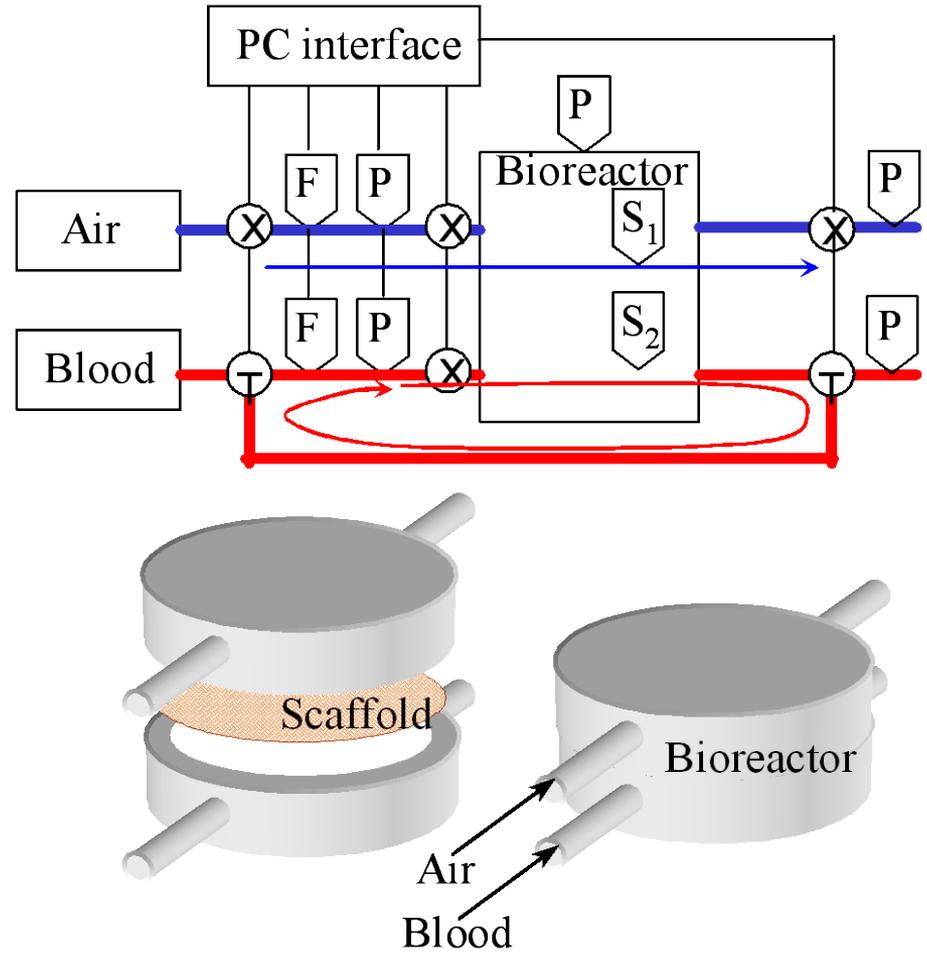




### Human lung structure



### In-vitro lung structure



Schematics of human lung and *in-vitro* lung structures. A bioreactor which simulates alveolus compartment is hooked up with gas sensors (S1) and blood gas sensors (S2). Flow sensors (F) and pressure sensors (P) are used for feedback control of air and blood streams.



## Can Barrier Models be Incorporated with $\mu$ CCA?

- Examples of important barrier tissues:
  - Gastrointestinal (GI) Track
  - Lung Epithelium
  - Skin
  - Blood Brain Barrier
- Barriers control entry of drugs into systemic circulation or into specific tissues (e.g. brain)
- To mimic oral uptake, inhalation, or adsorption, need barrier/systemic circulation model



# GI Tract Model

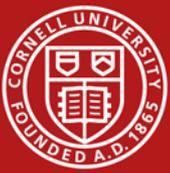
- Simulate oral adsorption of drugs and particles; combine with systemic model
- Three cell types: Caco-2 cells (epithelial); HT29-MTX (goblet or mucus producing cells); M cells (Caco-2 plus Raji B lymphocyte co-cultures)

See: Nature Nanotechnology, Mahler, et al., 2012, DOI: 10.1038/NNANO.2012.3



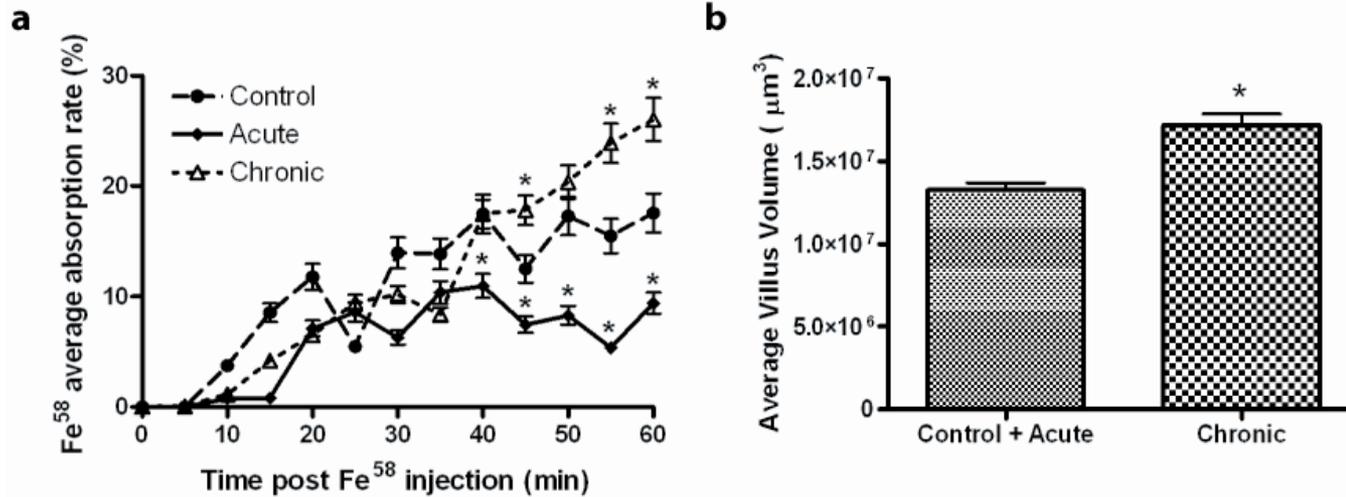
# In Vitro Nanoparticle Uptake Studies

- Particles transported differently dependent on size. The 50 nm particles through diffusion in membrane (non-energy requiring) and 200 nm particles through vesicles (energy requiring process)
- At moderate exposure ( $2 \times 10^9$  50 nm particles /mL or  $1.25 \times 10^{10}$  200 nm particles/mL) of fluorescent polystyrene carboxylated particles interfere with iron transport (reduce by 25 to 35%); could lead to iron deficiency
- Transport of 50 nm particles, across barrier alters effective “size” (only single particles, not clusters, transported) and surface charge of particles in systemic circulation



# Nanoparticles and in vivo GI Tract

1. In vivo (in chicks) nanoparticle exposure in short term inhibits iron transport (by 40%)
2. In vivo chronic exposure—chicks increase macro and microvilli area to regain normal iron transport
3. In vitro results (human cells) correlate with in vivo, observation, (chick) in short term



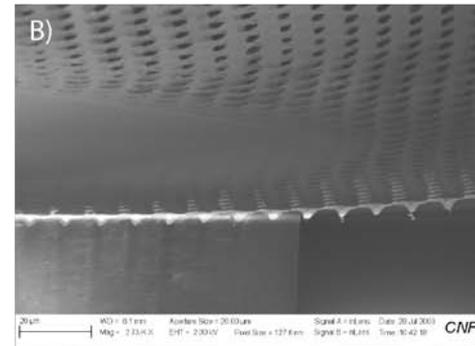
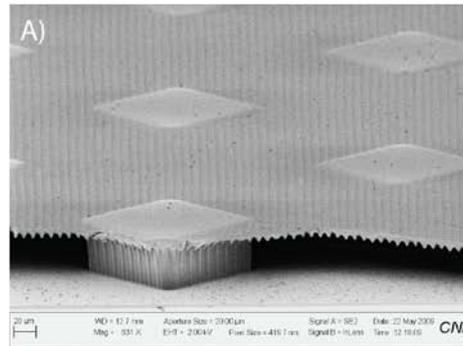
**Figure 5.** *In vivo* iron transport, liver ferritin, gene expression, and villus volume results.

(a) Duodenal loop iron absorption rate for control, acutely exposed and chronically exposed chickens. Blood samples were collected before stable isotope injection and then every 5 min and for 120 min post solution injection. (b) Average duodenal villus volume.

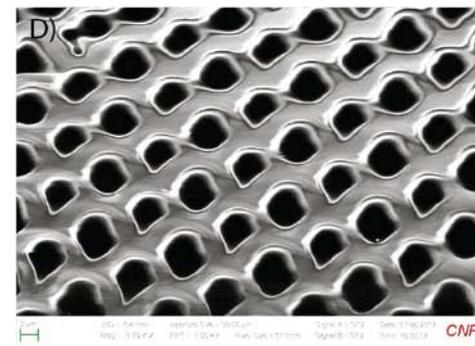
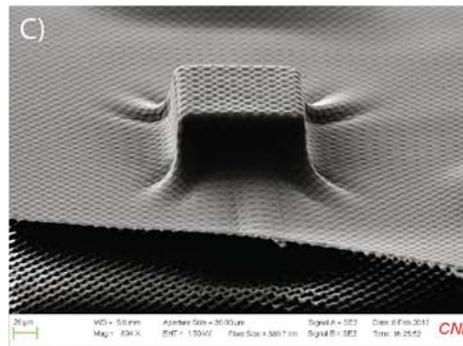


# Polymer Membranes with Pores for Microfluidic Cell Culture

**FLAT MEMBRANE** | SU-8 support posts create space for the basolateral chamber



**3D MEMBRANE** | Membranes are dried over silicon posts

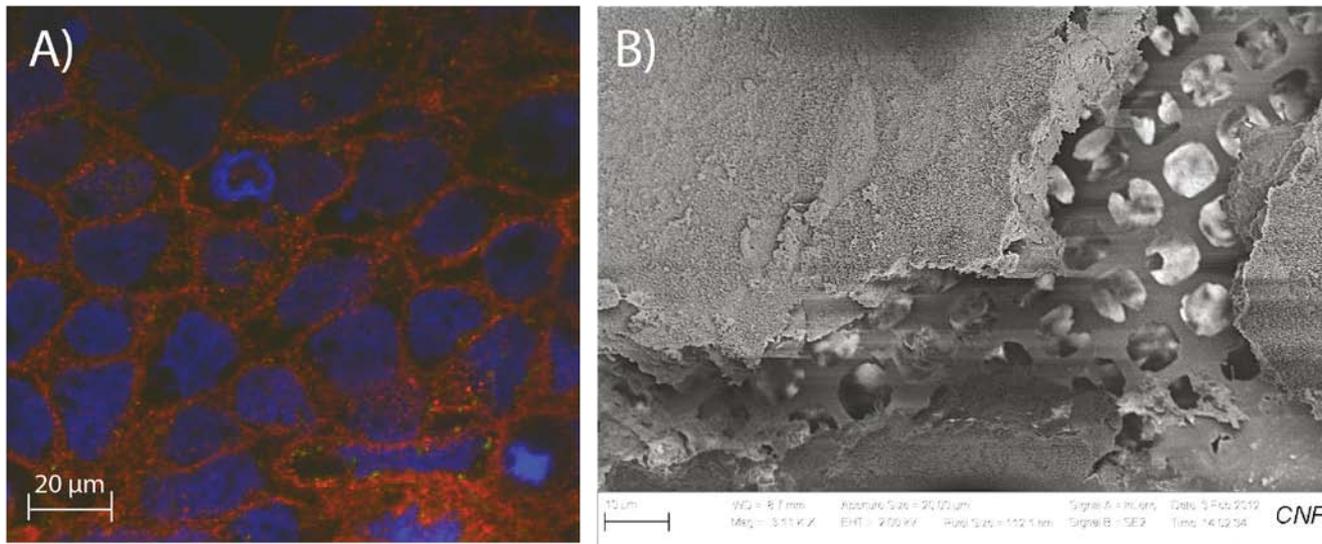


**Porosity: up to 40%. Pore sizes range from 0.5 to 4 μm.**  
**3D structures have aspect ratios of up to 4:1 (50 μm wide and 200 μm high).**



# SU-8 Membranes Support Caco-2 Cell Growth

**CACO-2 CELLS** | (A) Immunostained for occludin (red) and nucleic acids (blue).  
(B) Scanning electron microscopy image of porous membrane with Caco-2 cells.



**Caco-2 cells establish tight junctions.**

**The mechanical properties of the membrane support cell growth.**

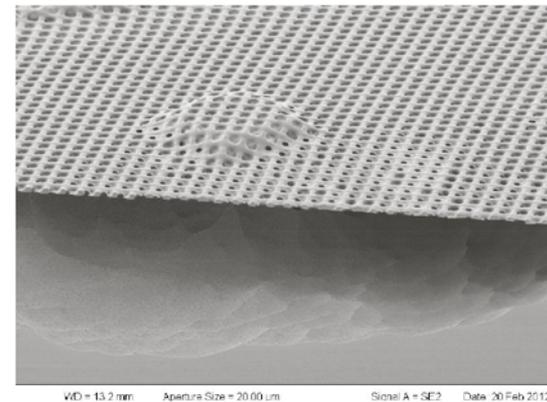
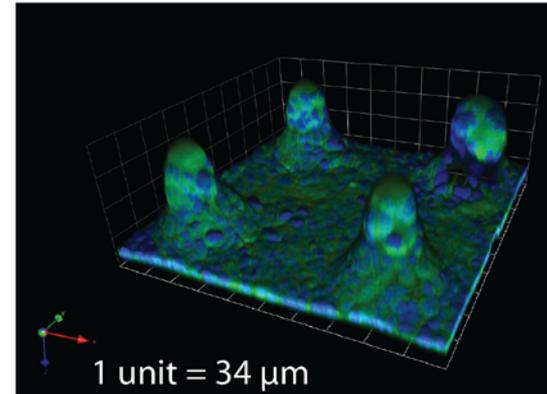
**The membranes are stable enough to withstand forces exerted by the cells.**



# Mimicking Key Aspects of the Gastrointestinal Tract Epithelium

**CACO-2 CULTURE ON 3D MEMBRANE** | Cells were grown for 21 days and covered the entire surface, including the villus tips

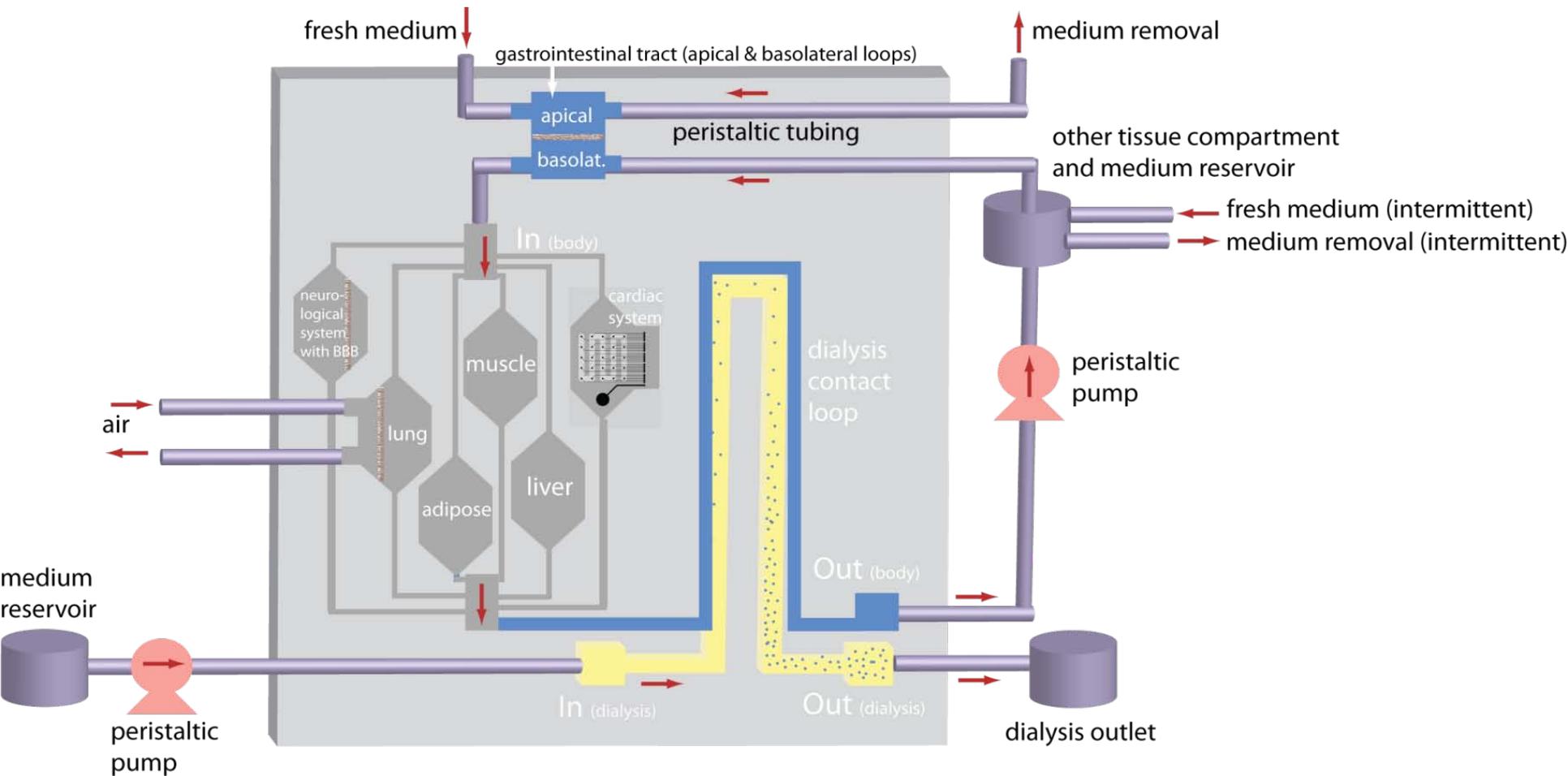
**3D MEMBRANE** | Silicon pillars can be removed with xenon difluoride that accesses the silicon through the membrane's pores.



**Caco-2 cell culture on 3D membranes mimicks key aspects of gastrointestinal villi. Access to basolateral side can be created by removing sacrificial silicon.**



# Prototype Human on a Chip





# Body-on-a-Chip

- PBPK model linked to physical device
- Realistic predictions for specific questions
- Potential low cost, high throughput technology
- Barrier tissues can be coupled to systemic circulation
- Authenticity will increase as more realistic, functional tissue constructs are integrated



# BLANK

- SLIDE
- FOR
- FORMATTING



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