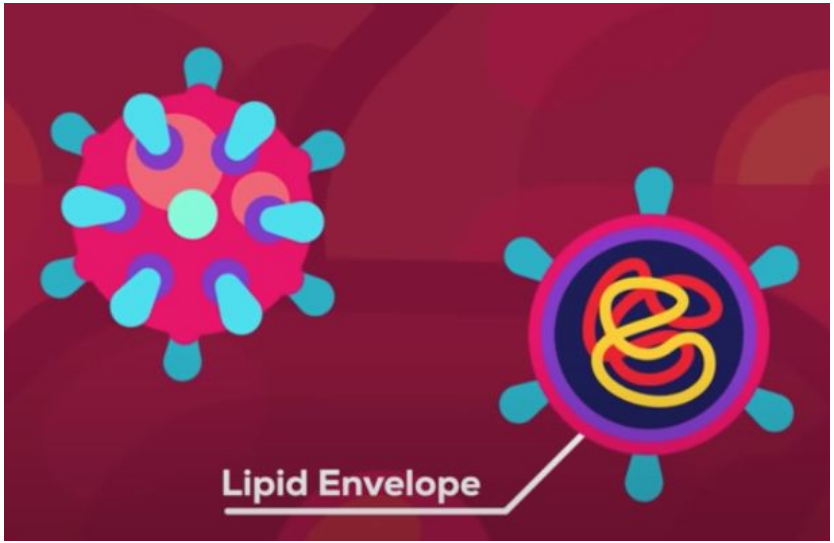
Organ-on-a-Chip Model for COVID-19

Matthew, Vincent, Emma and Sara

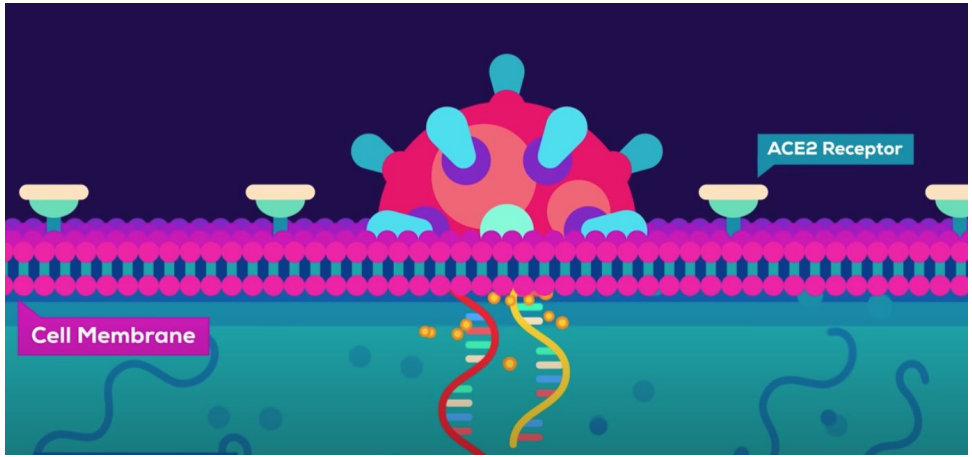
Background: COVID-19

- Coronavirus family: single-stranded RNA viruses
  - COVID-19: surrounded by lipid envelope with spiked proteins
- Characterized by: fever, cough, and other constitutional symptoms
- Mainly spread through droplet infection
- Unknown how long it can survive on surfaces



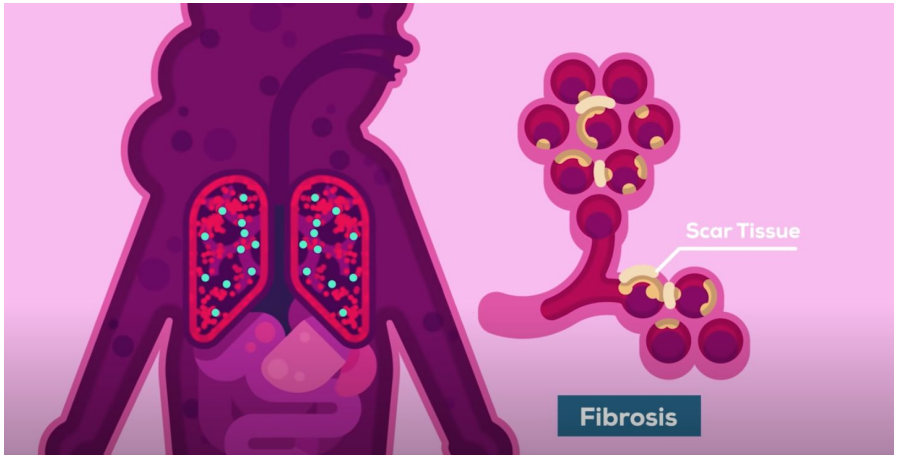
The Lungs

- Lined with epithelial cells as a protective layer for alveoli (air sacs)
- Membrane of epithelial cells contains ACE2 Receptors, which connect to coronavirus to transmit genetic material
    - Lower airways contain more ACE2 Receptors
- Genetic material adopted by epithelial cells, instructions to replicate, cell destruction releases more copies of the virus to infect other cells



Immune System

- Works to fight infected epithelial cells
- Communicates via cytokines
- Overactive immune response causes killing of both healthy and infected cells
- Cytokine storm -> killing of too many epithelial cells -> alveoli more susceptible to infection by bacteria -> pneumonia -> death



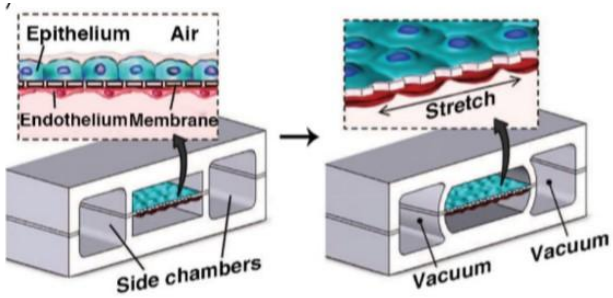
Current Gaps in COVID-19 Research

- Environmental effects on COVID-19 (such as weather and temperature)
- Survival on surfaces
- Transmission factors (ie. food, alternate hosts)
- Treatments
- Vaccinations

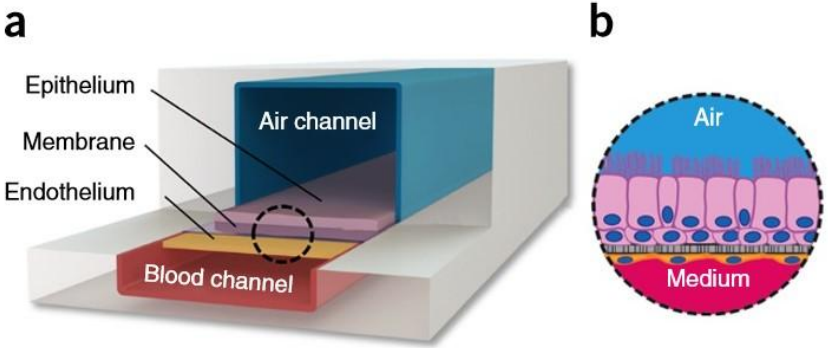
Why Organ-on-a-Chip Model for COVID-19?

- Difficult to parse mechanisms of disease pathology in human clinical trials, which are still not well understood
- Clinical trials often more expensive and can have longer timescales
- Single cell *in vitro* models don't recapitulate *in vivo* complexity
    - Particularly lung airway-microvascular interface
- Can analyze cellular effects both individually and collectively
- Animal models may not match human physiology

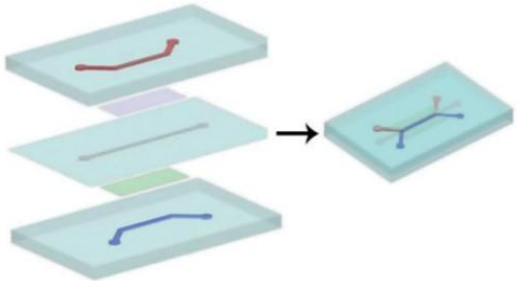
Lung-on-a-Chip Models



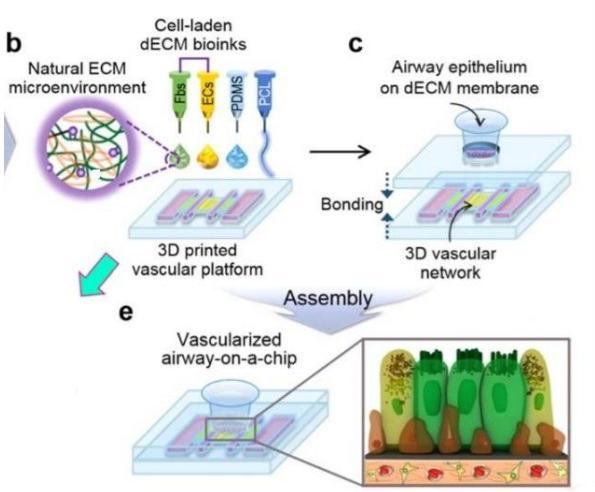
Huh et al., 2010



"Small airway-on-a-chip", **Benham et al., 2015**

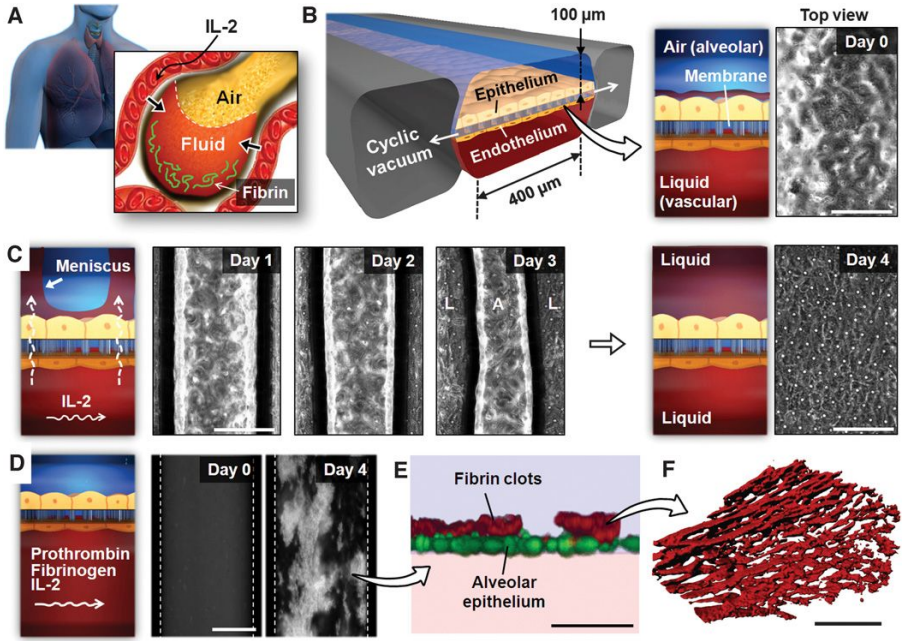


Sellgren et al. ,2014

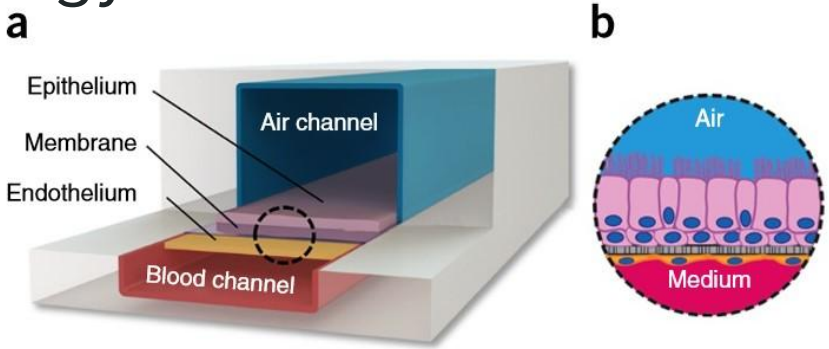


Park et al. 2018

Lung-on-a-chip models: Pathology



Pulmonary Edema Model. **Huh et al., 2012.**



"Small airway-on-a-chip", **Benham et al., 2015**

Viral mimic: Polyinosinic polycytidylic acid (poly(I:C)) **Benham et al. 2016**

Human Rhinovirus-induced Asthma Model. **Villenave et al., 2017**

Current Limitations/Innovation

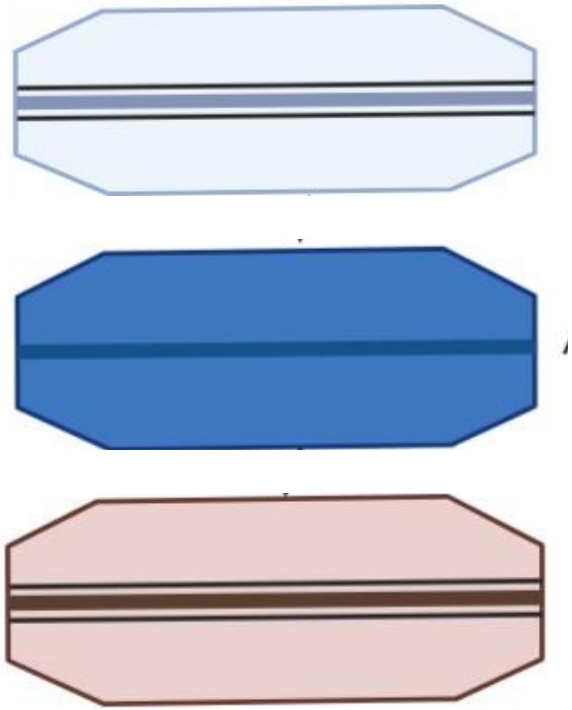
- Potential hydrophobic drug absorption from PDMS
- Membrane interface is often limited
  - Polyester needs to be coated
  - Typically too thin(basement membrane >>>10 microns)
  - Doesn't match ECM mechanical properties
- Some systems (e.g. Benam et al.) don't include a breathing mechanism
- No simulation of upper respiratory tract
- **__**To date, there is no current COVID-19-specific application of lung-on-a-chip or organ-on-a-chip__**

COVID-19 Organ-on-a-Chip: Design Parameters

Microfluidic chip material	Cells	Membrane material	Breathing mechanism	Channel design
PDMS w/ PEG-grafted channels	• Human airway epithelial cells • Human pulmonary microvascular endothelial cells	Matrigel+ Type I Collagen	Apply cyclic vacuum to hollow channels (10% cyclic strain at 0.2 Hz)	Two separate chips in series. One with a single wider channel**(Upper Respiratory Tract or URT)** and one with two branched smaller channels **(Lower Respiratory Tract or LRT)**
Biocompatible, optically transparent, **avoids absorption of small molecules**, tunable mechanical properties	Primary cells are preferable, and been used in previous lung-on-a-chip models	Matrigel+Type I Collagen= Better cell attachment and ECM mimicry.	From **Huh et al. 2012**.	Simulates upper and lower respiratory tracts.

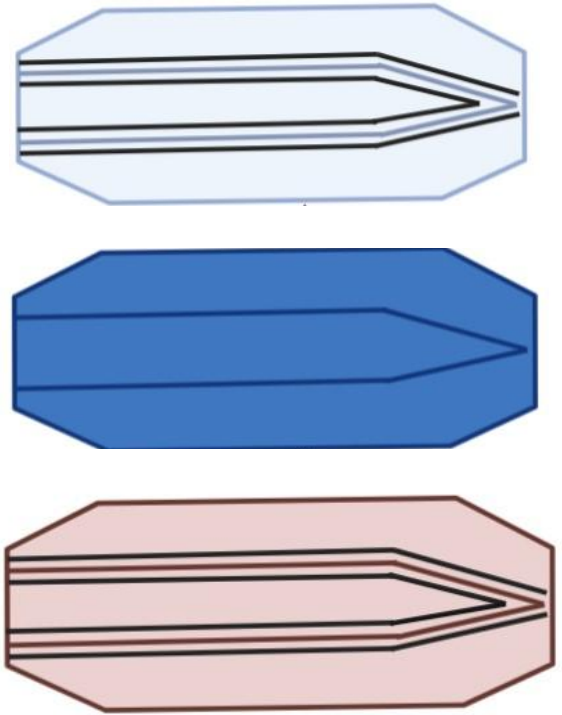
COVID-19 Organ-on-a-Chip: URT Device Fabrication

- Stereolithography to make the chip parts out of PDMS with specified channels
- **Epithelial chip** has one large channel with breathing channels
  - Main channels➔ **4 mm wide x 1 mm high**
  - Breathing channels➔**1 mm wide x 1 mm high**
- **Hydrogel chip** has one large channel
  - **3 mm wide x 0.5 mm high**
- **Bottom chip** has one large channel with breathing channels
  - Main channels➔ **4 mm wide x 0.2 mm high**
  - Breathing channels➔**1 mm wide by 0.2 mm high**
  - No cells with be seeded on these chip channels, just for media flow to the other chip



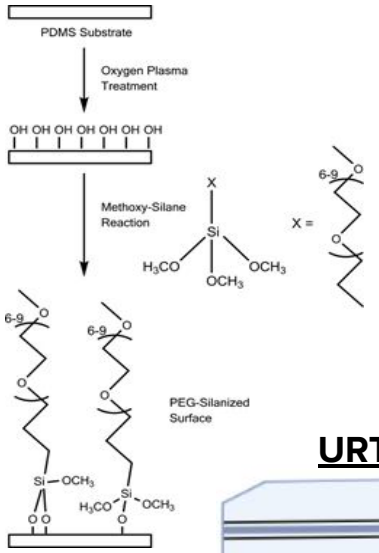
COVID-19 Organ-on-a-Chip: LRT Device Fabrication

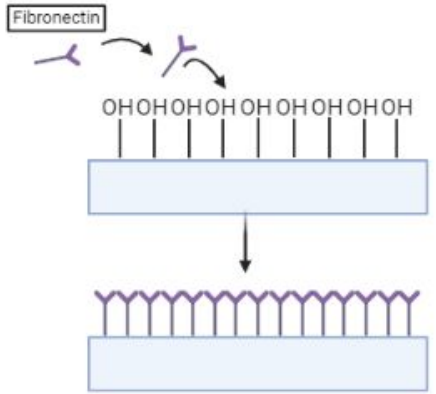
- Stereolithography to make the chip parts out of PDMS with specified channels
- **Epithelial chip** has two small branched channels with breathing channels
  - Main channels➔ **1.5 mm wide x 1 mm high**
  - Breathing channels➔ **1 mm wide x 1 mm high**
- **Hydrogel chip** has two small branched channels
  - **1 mm wide x 0.5 mm high**
- **Endothelial chip** has two small branched channels with breathing channels
  - Main channels➔ **1.5 mm wide x 0.2 mm high**
  - Breathing channels➔ **1 mm wide by 0.2 mm high**

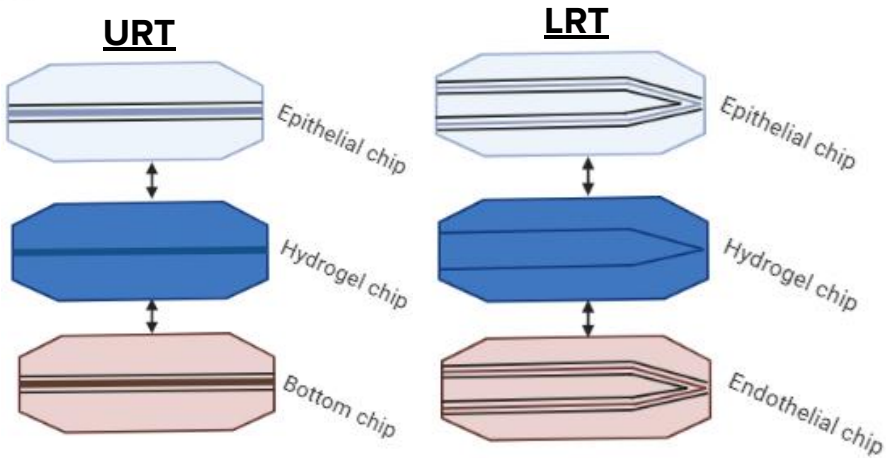


COVID Organ-on-a-Chip: Chip Modification and Assembly

- Oxygen plasma treat all three chip pieces (both URT and LRT)
  - **PEG-Silane** into **epithelial** and **endothelial channels**→ PEG grafting
  - **Fibronectin** coating for **hydrogel channel**
    - Better hydrogel adhesion
  - Bind three microfluidic pieces together after channel modifications(both URT and LRT)







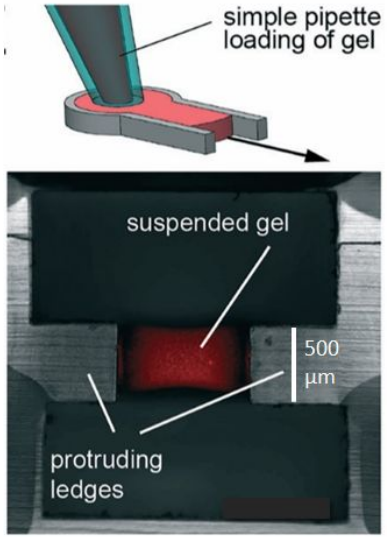
COVID-19 Organ-on-a-Chip: Chip Modification (cont'd)

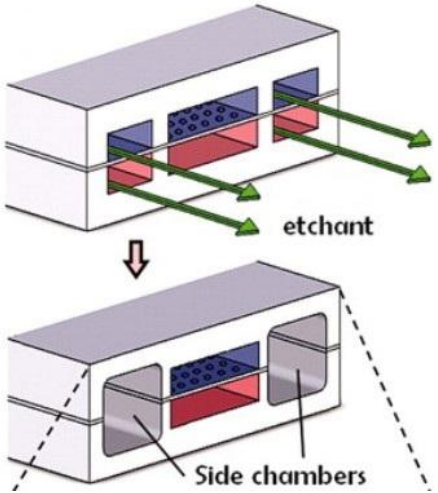
Hydrogel Insertion

- Inject Matrigel+Type I Collagen gel into fibronectin coated middle channels
- Polymerize at 37°C for 1 hour
- Hydrate overnight with media

Hollow Channel Development

- Flow etchant solution through breathing chambers to remove PDMS layer from the middle hydrogel chip.
  - Tetrabutyl-ammonium fluoride
  - N-methylpyrolidinone





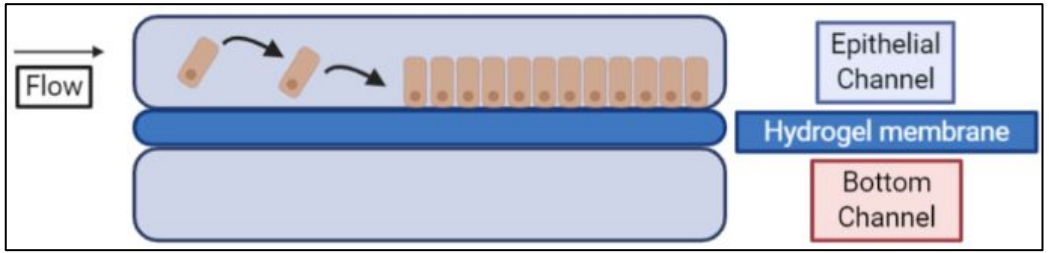
COVID-19 Organ-on-a-chip: URT Tissue Development

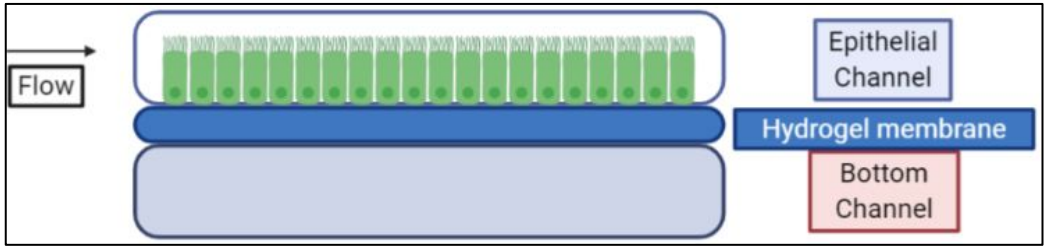
Seed epithelial cells

- **3.5x10^5 cells/cm^2 seeding density** on hydrogel membrane
- Constant flow through both epithelial endothelial for 4-5 days to reach confluency

Epithelial Cell Differentiation

- Remove liquid from top channel to create air-liquid interface
- **Differentiation into mucociliary epithelium** at 3-5 weeks
- Some squamous differentiation may occur





COVID-19 Organ-on-a-Chip: LRT Tissue Development

Seed epithelial cells

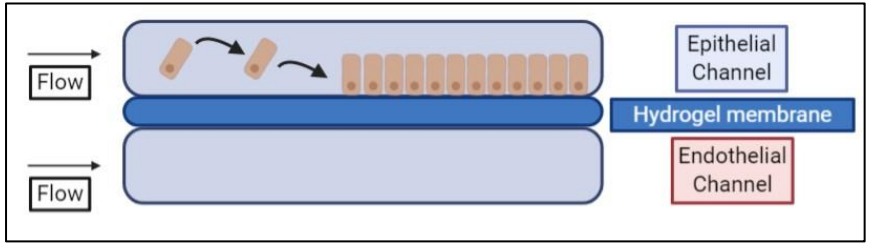
- **3.5x10^5 cells/cm^2 seeding density** on hydrogel membrane
- Constant flow through both epithelial endothelial for 4-5 days to reach confluency

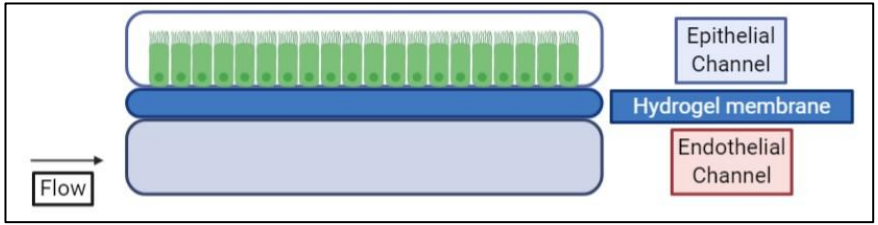
Epithelial Cell Differentiation

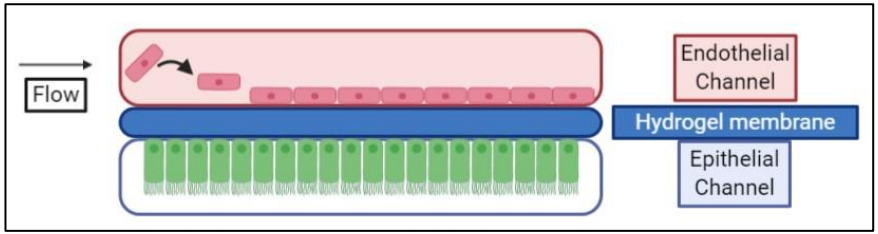
- Remove liquid from top channel to create air-liquid interface
- 3 ug/mL retinoic acid to bottom channel media to prevent squamous differentiation
- **Differentiation into mucociliary bronchiolar epithelium** at 3-5 weeks

Endothelialization

- After epithelial differentiation, seed **2 x 10^5 cells/cm^2 endothelial cells** on bottom channel surface
- Confluency after 3-6 days of media flow



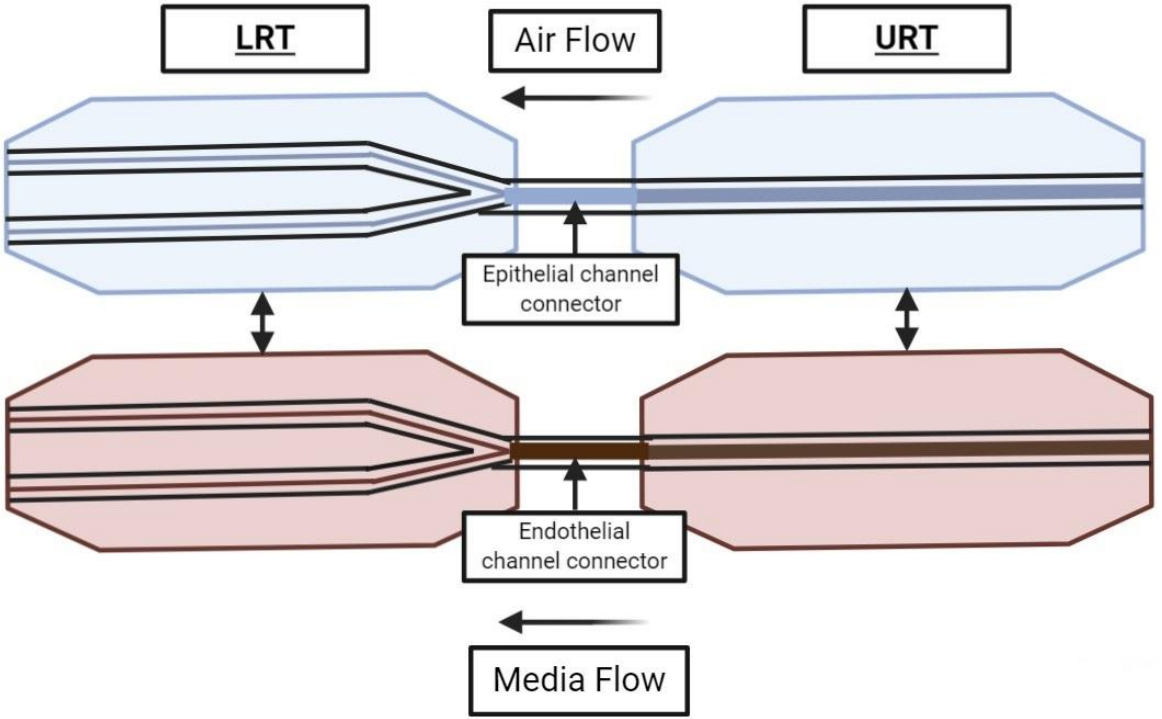




COVID-19 Organ-on-a-chip: Synthesis

- After cell seeding and differentiation, connect URT and LRT microfluidic systems
  - Fluid connections via sterile Tygon tubing
  - Vacuum connectors

- Maintain physiological flow rates for air and media(~100-200 µL/min)

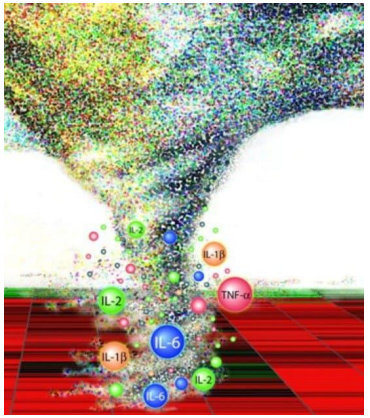


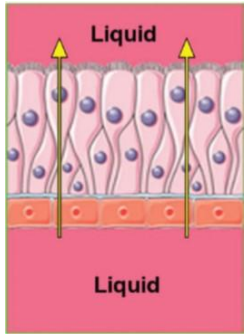
COVID-19 Organ-on-a-Chip: Validation

- Hoescht live/dead test
- Immunostaining for relevant cell markers
  - ACE2 receptors
  - β-tubulin IV (epithelial cilia markers)
  - Aquaporin 5
  - VE-cadherin
  - DAPI
- Barrier integrity test
  - FITC-dextran test, as seen in Sellgren et al.

COVID-19 Organ-on-a-Chip: Applications

- Studying early onset effects of the infection
  - COVID-19 effects on lung system
    - Effects of factors from cytokine storm (e.g. Il-1, Il-6, Il-17)
    - Edema (presence of fluid in epithelial barrier)
    - Change in barrier function
    - Gas transport across alveolar-capillary barrier
  - Identifying disease biomarkers
- Screening RNA vaccines
    - Direct transfection to the cells
    - Production of RNA/DNA vaccines from infected cells





COVID-19 Organ-on-a-Chip: Limitations

- Requires two separate chips
- Not including a lymphatic system for drainage
- Doesn’t account for potential systemic effects
  - E.g. Liver
- Bronchioles can be as large as 5 mm
- Does not measure impact of virus on immune cells; only impact of immune response on lung cells

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Questions?