Etiology of COPD and In Vitro Models

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Outline: Etiology of COPD

Part 1. Overview of COPD

1. Definitions of COPD
2. Medical manifestations/disease states encompassed
3. Risk factors, exacerbations, & comorbities
4. Responses to tobacco smoke inhalation
5. Examples of bronchitis & emphysema
6. Summary of COPD etiology

COPD: Historical Definition

Patients afflicted with COPD can have one or more symptoms of chronic bronchitis, emphysema, or both. These individuals have increased susceptibility to infection and air pollution.

- Chronic bronchitis
  - Excessive mucous production
  - Airway wall thickening
  - Epithelial squamous metaplasia
  - Leukocyte recruitment
- Emphysema
  - Airspace enlargement
  - Parenchymal destruction
- Small airways disease (Prof. Dr. Dirkje S. Postma)
  - A collection of a wide variety of diseases affecting small airways

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COPD: Current Definitions

Global Initiative for Chronic Obstructive Lung Disease (GOLD)

"a common preventable and treatable disease characterized by persistent **airflow limitation** that is **usually progressive** and associated with an enhanced **chronic inflammatory response** in the airways and the lungs to **noxious particles or gases**. Exacerbations and comorbidities contribute to the overall severity in individual patients."

American Thoracic Society (ATS)/European Respiratory Society (ERS)

"Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease state characterized by **airflow limitation** that is not fully reversible. The airflow limitation is **usually progressive** and is associated with an abnormal **inflammatory response** of the **lungs to noxious particles or gases**, primarily caused by cigarette smoking. Although COPD affects the lungs, it also produces significant systemic consequences."

- **Progressive (usually) airflow limitation in airways/lungs due to noxious particles or gases and associated with inflammatory response**

COPD: Risk Factors, Exacerbations, & Comorbidities

Risk Factors

- Host:
  - Genetic
    - α_1 antitrypsin deficiency (Decramer 2012),
    - numerous other genes implicated (D-G&M 2014;)
  - Gender (male dominated (D-G&M 2014)
  - Airway hyperreactivity, IgE and asthma

- Exposure:
  - **Smoking (main risk factor: active and passive)**
  - Socio-economic status
  - Occupation
  - Environmental pollution
  - Perinatal events and childhood illness
  - Recurrent bronchopulmonary infections
  - Diet

Exacerbations (>70% bacterial or viral infections)

Comorbidities: Cardiovascular disease, Muscle weakness, Hypertension, Osteoporosis, Lung cancer, Anxiety/depression

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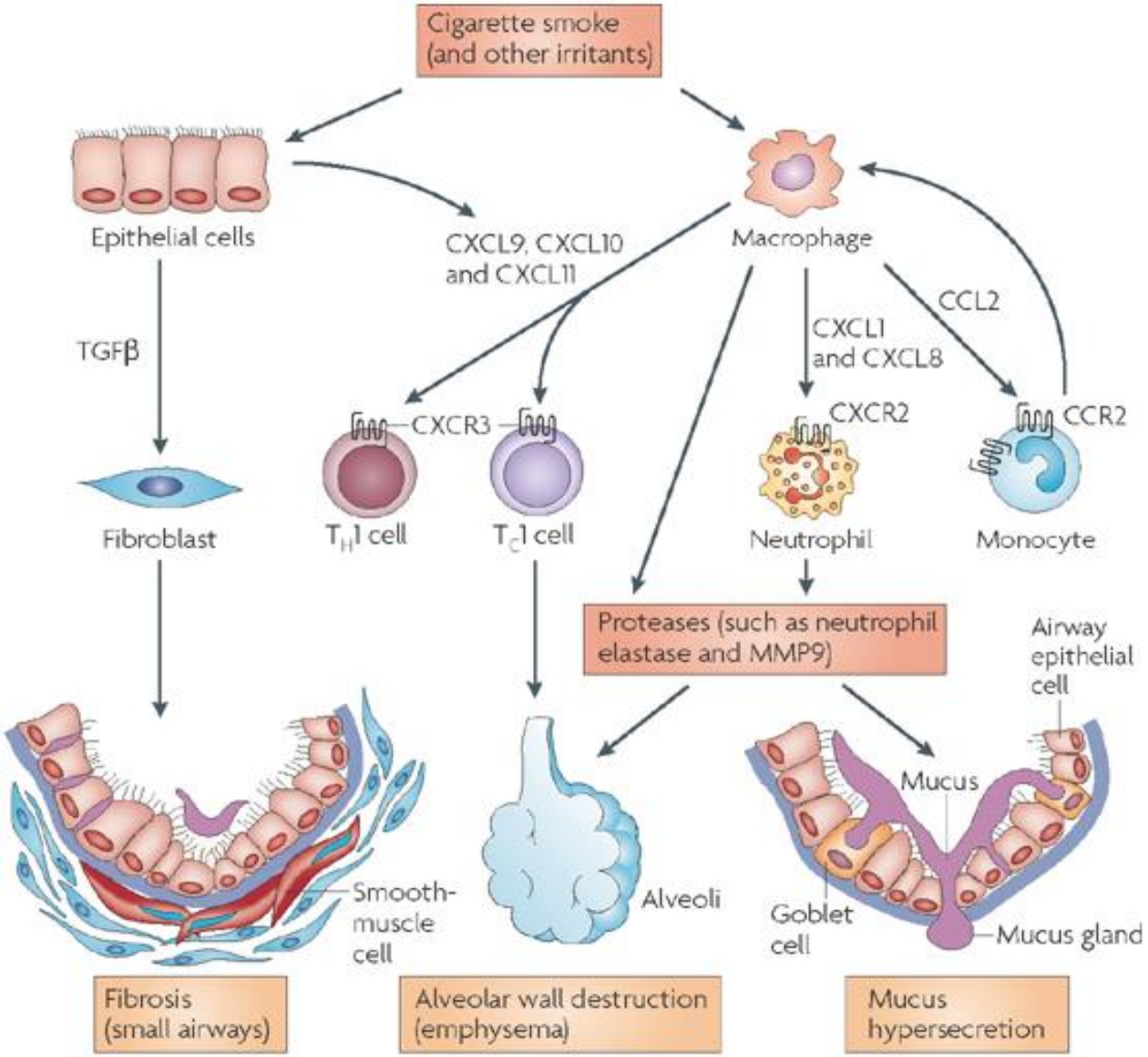
Schematic of Tobacco-COPD events

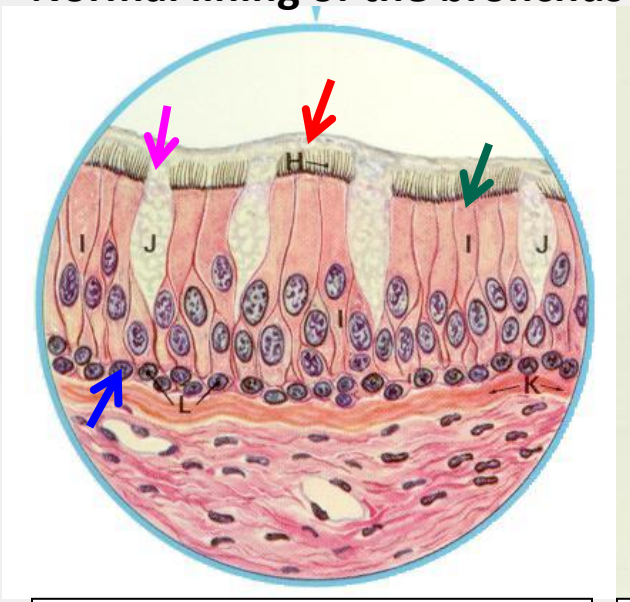


Nature Reviews Immunology 8, 183-192 (March 2008) Peter J. Barnes

http://www.nature.com/nri/journal/v8/n3/full/nri2254 Nature Reviews | Immunology

Tobacco Smoke Exposure: Changes in Lining of the bronchus

Normal lining of the bronchus



H: Cilia – sweep mucous & particulates

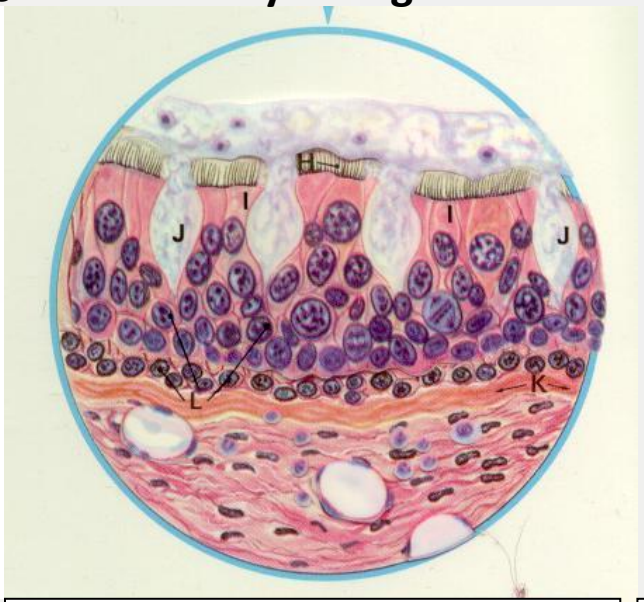
I: Columnar cells – yield cilia

J: Goblet cells – produce mucous

L: Basal cells – comprise bottom layer

Illustration & Excerpt Source: WhyQuit.com

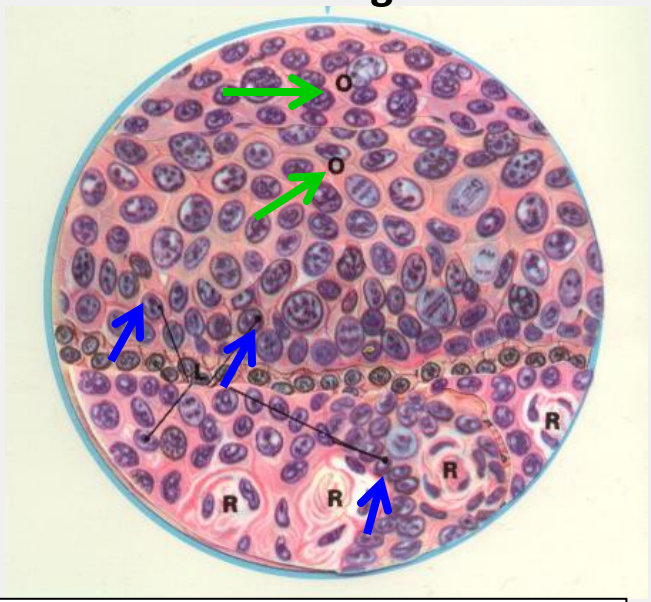
Early changes



- **columnar cells** are starting to be **crowded** out and displaced by **additional layers of basal cells**
- **fewer cilia are present** and are functioning at a much **lower level of efficiency**
- **chemicals in tobacco smoke are toxic to cilia**, first slowing them down, soon paralyzing them all together and then destroying them.

Illustration & Excerpt Source: WhyQuit.com

Later changes



- **ciliated columnar cells are totally displaced.**
- **smoker is more prone to infection** from the loss of the cleansing mechanism of the cilia
- **O: abnormal cells are cancerous squamous cells.** These cells will eventually **break through the basement membrane wall and invade** into underlying lung tissue
- *Illustration & Excerpt Source: WhyQuit.com*

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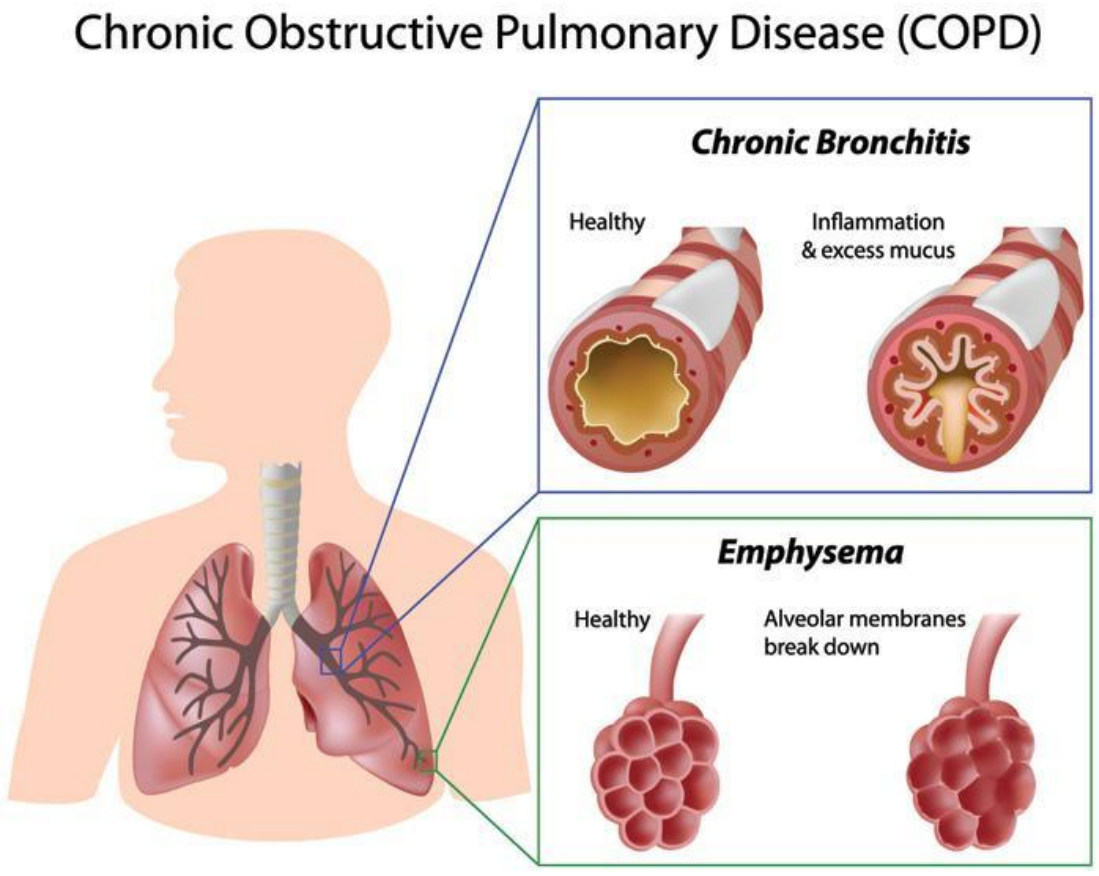
COPD: Chronic Bronchitis, Emphysema & Small Airways Disease



http://www.livweltherapeutics.com/area-copd.html

Small Airways Disease:

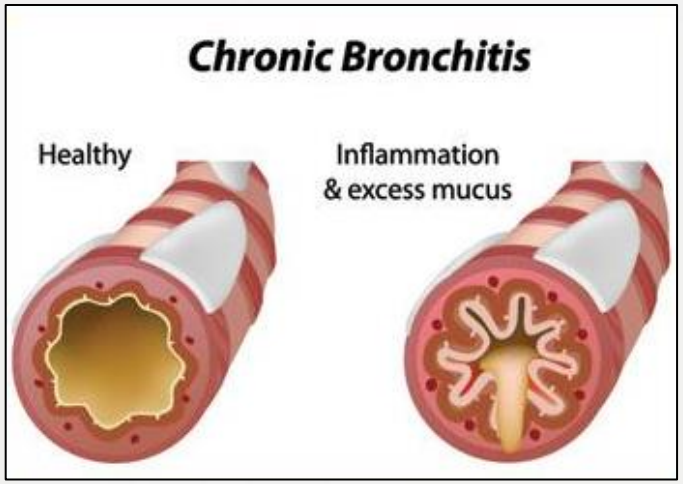
Include a wide variety of diseases often including a form of bronchiolitis

- Bronchiolitis = a variety of inflammatory conditions involving the small airways
- Bronchiolar and peribronchiolar inflammation may be focal or diffuse and may or may not be associated with scarring and bronchiolar metaplasia.
- Bronchiolar mucosa may undergo bronchiolar, squamous, or goblet cell metaplasia.

Timothy Craig Allen (2010) Pathology of Small Airways Disease. Archives of Pathology & Laboratory Medicine: May 2010, Vol. 134, No. 5, pp. 702-718.

http://www.archivesofpathology.org/doi/full/10.1043/1543-2165-134.5.702

Chronic Bronchitis

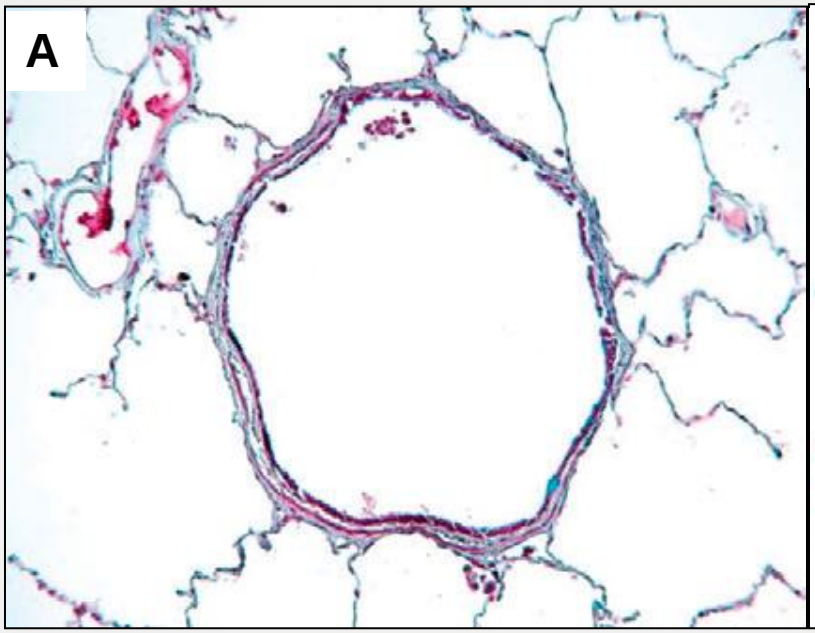


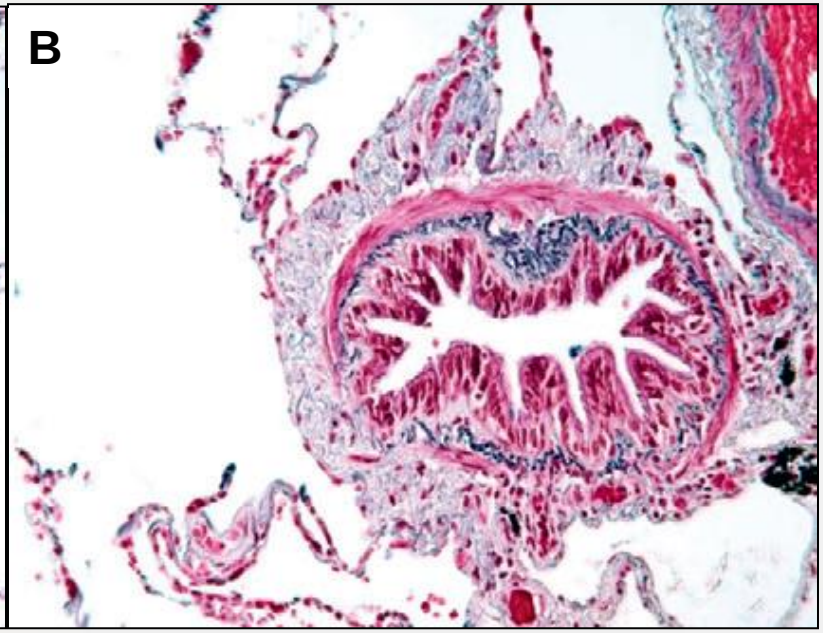
http://www.livweltherapeutics.com/area-copd

Comparison of airway features in a healthy individual and in a patient with chronic obstructive pulmonary disease

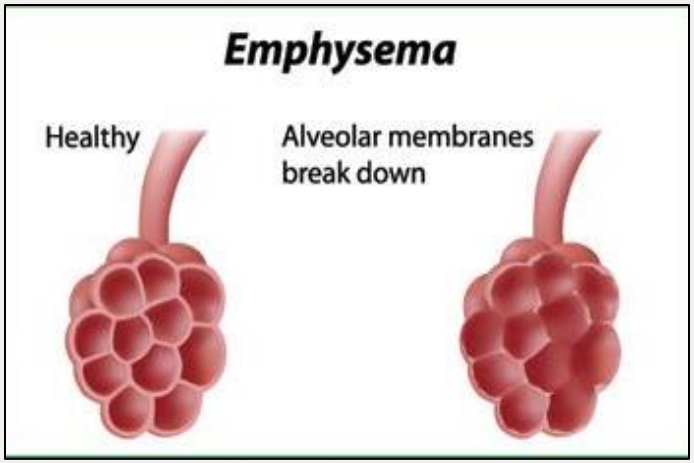
A: Normal airway.
B: In COPD, airways are narrowed by infiltration of inflammatory cells, mucosal hyperplasia, and deposition of connective tissue in the peribronchiolar space.

Hogg JC, Timens W. The pathology of chronic obstructive pulmonary disease. Annu Rev Pathol 2009; ***4:*** *435–59.*





Emphysema

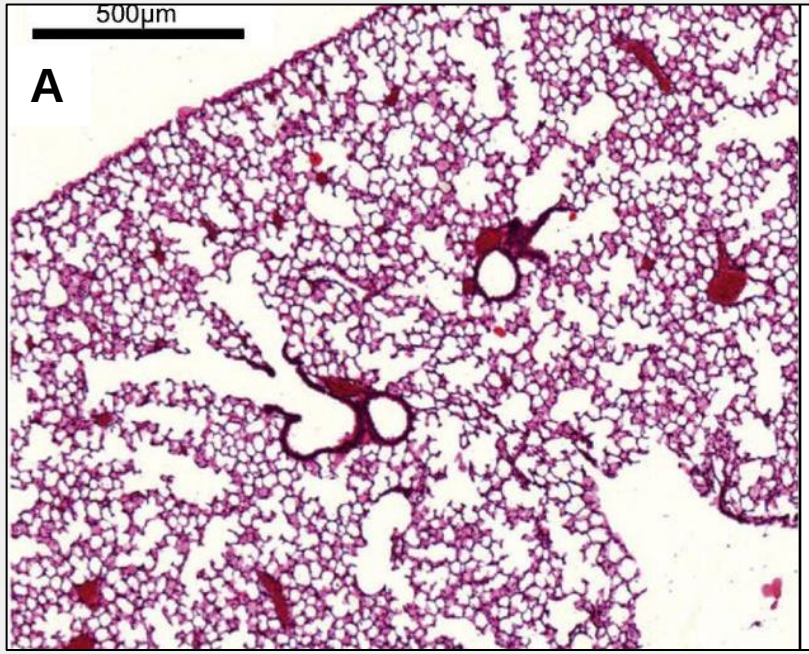


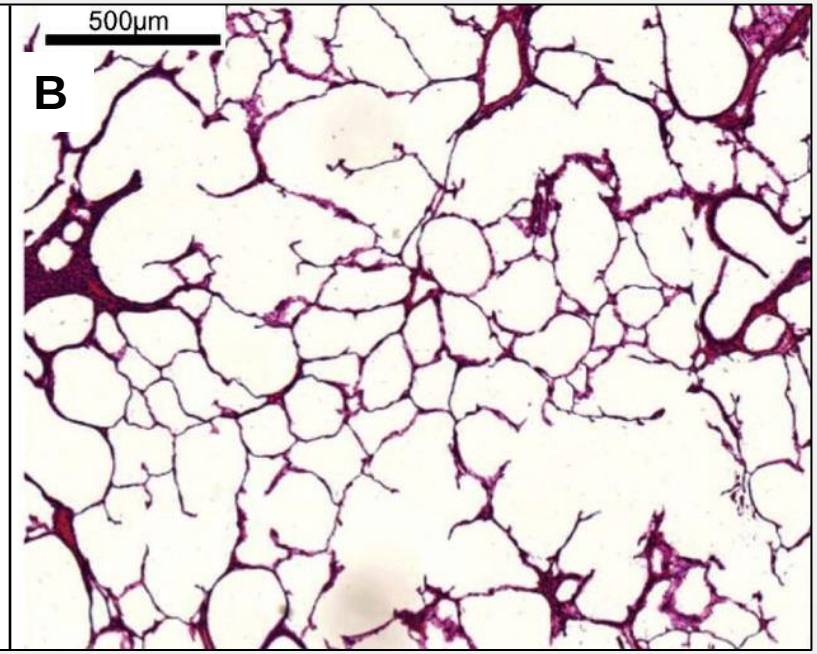
http://www.livweltherapeutics.com/area-copd

Comparison of airway features in a healthy individual and in a patient with chronic obstructive pulmonary disease

A: Normal airway.
B: Emphysema: Septal collapse is evident. These changes are permanent and cause a decrease in number of alveoli, an increase in size of alveoli, and most importantly, a net decrease in the surface area available for gas exchange.

http://www.headingfortheexits.com/emphsema-can-kill-you-because-planes-fly/





Chronic Obstructive Pulmonary Disease (COPD)

Initiation, Progression, & Manifestation of COPD

Initiating event:

Tobacco exposure or other toxic insult to lung epithelium

1. Ligand-receptor interactions

2. Intracellular response

3. Oxidative stress

4. Initiation of autocrine, paracrine, and endocrine signaling

5. Cellular damage

Tissue Response:

1. Cytokines/chemokines

2. Increased integrin and adhesion molecule expression

3. Monocyte recruitment (persistent influx of neutrophils)

4. Protease/antiprotease imbalance

5. Adverse cellular ion homeostasis-dehydration

6. Oxidative stress

7. Inflammation

Tissue Effects:

1. Ciliary dysfunction

2. Increased mucous secretion

3. Fibroblast activation

4. Goblet cell hyperplasia

5. Bronchial epithelial squamous metaplasia

6. Narrowing of airways

7. Collagen deposition

8. Parenchyma/tissue destruction

9. Injury/repair cycling

Pulmonary Effects:

1. Reduced lung elasticity

2. Reduced airflow

3. Airspace enlargement

4. Small airway remodeling

5. Vascular remodeling

6. Hyperinflation

7. Chronic inflammation

8. Fibrosis

Clinical manifestations:

1. Chronic bronchitis

2. Emphysema

3. Small Airways Disease

4. Increased susceptibility to infection and air pollutants

COPD:

Progressive (usually) airflow limitation in airways/lungs due to noxious particles or gases and associated with inflammatory response

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Outline: In Vitro Models

Part 2. Overview of In Vitro Pulmonary Models

1. Introduction to In Vitro/Ex Vivo models

2. Types of models currently used in mainstream research
   - Cell lines
   - Primary cells
   - 3D airway cultures
   - Ex vivo tissue

3. Important considerations in choice of model

4. Upcoming Technologies

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In Vitro/ex vivo Models

- A host of in vitro/ex vivo pulmonary models are available
- Used for a multiplicity of applications including:
  - Drug development
    - Efficacy
    - Adverse effects
  - Assessment of environmental toxicants
  - Personal care & cosmetics product development
  - Etc.

- For this workshop, a focus on models and assays that have demonstrated fit for purpose
  - Suitability in detecting one or more components in COPD etiology
  - Commercially available

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In Vitro Models for COPD

1. Cell lines: immortalized cells
   - Immature, transformed or cancer cells that have the capacity to expand and (possibly) mature to some degree
2. Primary cells
   - Derived from normal or diseased tissues but may not have capacity to expand greatly in number (e.g. limited supply)
3. 3D cultures/tissues
   - Reconstructed airway epithelium
4. Ex vivo tissues
   - Precision cut lung slices (PCLS)
5. New technologies:
   - Lung on a Chip
     - Wyss Institute: Dr. Donald Ingber
     - RPI & UNC

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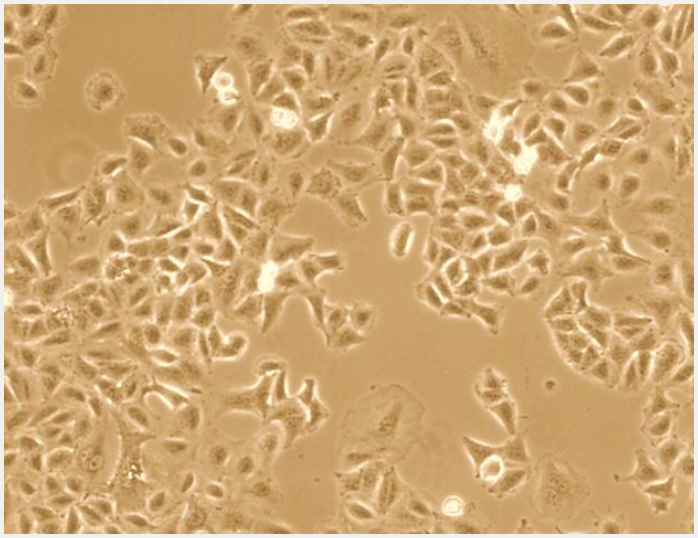
1. Cell Lines

Advantages:

- Economical means to generate cell based data
- Typically very reproducible
- Usually straightforward to use and easy to culture
- Can be easily expanded, cryostored, and banked for later use

Disadvantages:

- Not considered as physiological as primary or 3D models
- Are transformed or derived from cancerous tissue
- Passage “drift” can occur



A549 cell line

http://www.invitro.de/bildergalerie.html

E.g. H292: mucoepidermoid carcinoma origin

BEAS-2B: adenovirus transformed bronchial epithelial cell line

NCI-H441: lung adenocarcinoma epithelial cell line

A549: adenocarcinoma of alveolar origin (lack of tight junctions)

The Cell Line NCI-H441 Is a Useful in Vitro Model for Transport Studies of Human Distal Lung Epithelial Barrier

*Johanna J. Salomon,† Viktoria E. Muchitsch,† Julia C. Gausterer,† Elena Schwagerus,† Hanno Huwer,‡ Nicole Daum,§ Claus-Michael Lehr,§ and Carsten Ehrhardt†,**

2. Primary Cells

Advantages:

- Not immortalized
- More representative of individuals in population
- Can be expanded, cryo-stored, and banked for later use, but donor variability impacts the quality of tissue

Disadvantages:

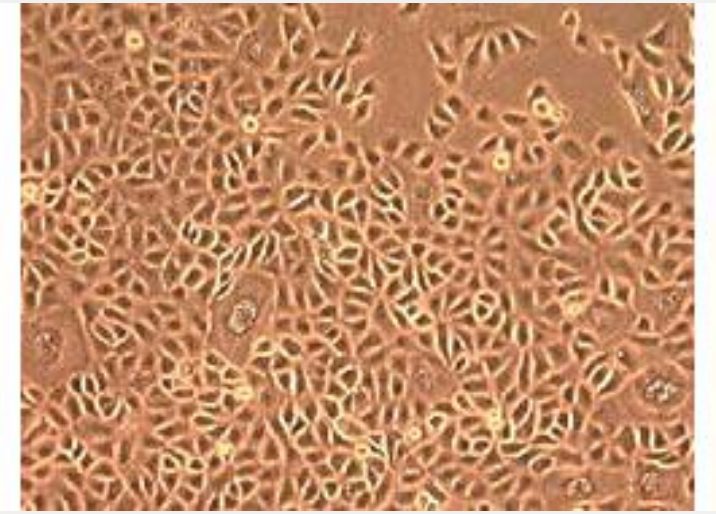
- Not immortalized and can be expensive
- Reproducibility is variable across different donors
- Can be difficult to culture and utilize

Cell origins:

- Tracheobronchial Epithelia
- Alveolar Epithelia
- Cells of disease states available

E.g.

- Normal human bronchial epithelial cells (NHBE)
- NHBE + fibroblast co-cultures



Human bronchial-tracheal epithelial cells (Lonza)

http://www.lonza.com/products-services/bio-research/primary-cells/human-cells-and-media/airway-cells-and-media/nhbe-normal-human-bronchial-tracheal-epithelial-cells.aspx

3. 3D Epithelial Cultures

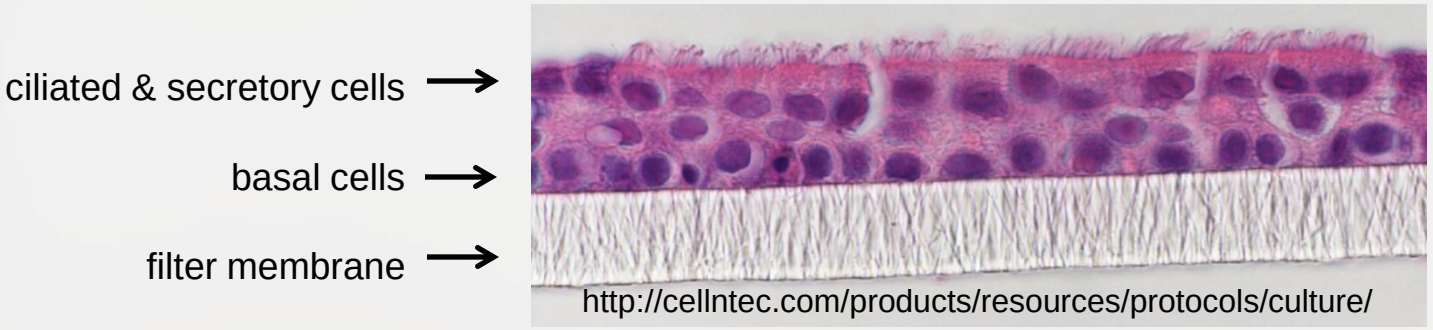
- 3D cultures can be created by expanding and differentiating primary epithelial cells at an air-liquid interface (ALI)
- Exposure to air on apical side of cultures, and medium on basal (filter) side allows for pseudo-stratification of cell layers, yielding multiple cell types

Advantages:

- More physiologically relevant
- More representative of individuals in population
- Allows study of cell types/functions that are not available in 2D models (e.g. ciliary beating)

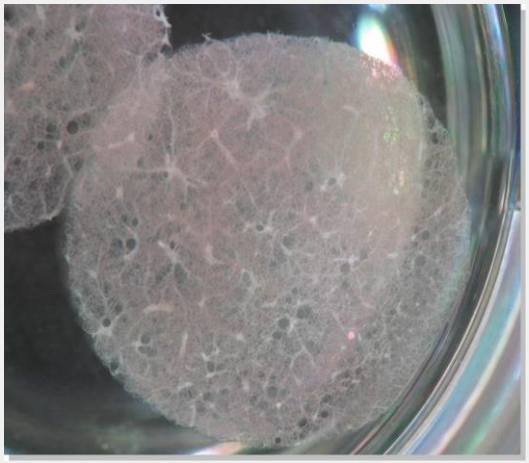
Disadvantages:

- Are relatively expensive compared to 2D models
- Reproducibility is variable across different donors
- Requires multi-week culture for product maturation



4. Ex Vivo Lung Tissues: PCLS

- Lung slices are created from whole lungs by inflating with agarose solution, coring gelled tissues, and slicing cores in a precision slicer
- PCLS (typically ~300-1000 µm thickness) sliced from cores can be cultured using ALI insert, roller drum method, or rocking platform
- PCLS can be cultured for days or weeks and are used for acute or chronic exposures and/or evaluation



Credit: BASF/Fraunhofer
http://www.item.fraunhofer.de/en/business_units_new/pre-clinical_pharmacology/Ex-vivo_methods.html

Advantages:

- Most (?) physiologically relevant of non-whole organ ex vivo models
- More representative of individuals in population
- Allows study of cell types/functions that are not available in other models
  - E.g. macrophages, airway contractility, etc.

Disadvantages:

- Availability of high quality tissue is infrequent, highly variable quality across donors
- Reproducibility is variable across different donors
- Labor intensive setup procedure by well trained staff required

Some Important In Vitro Model Considerations

- Cost
- Reproducibility
- Ease of use, accessibility
- Interlab transferability
- **Endpoints modeled**
- Tissue origin
  - If Human, how well does it translate to whole body?
  - If non-human, how well does it extrapolate to human?
- Amenable to high throughput
- Etc.…

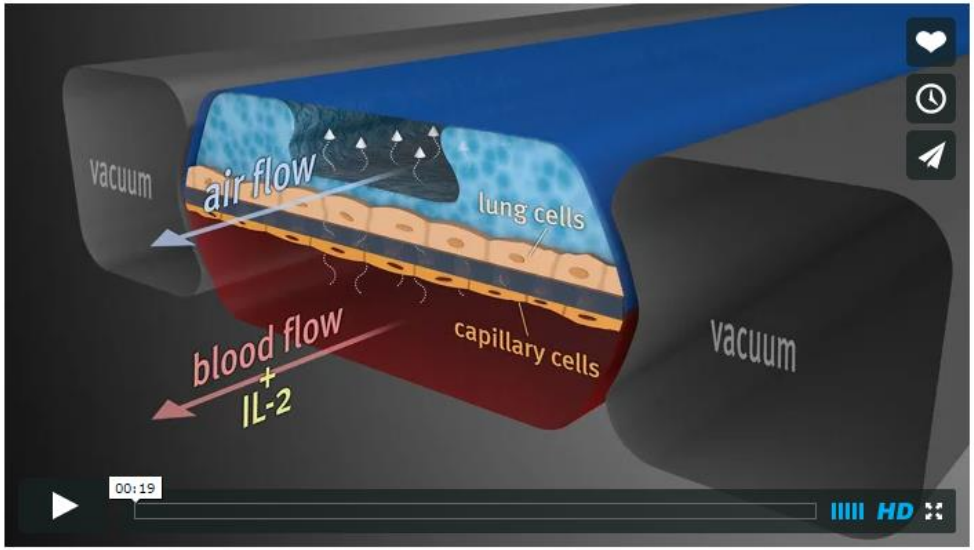
E.g. Inflammation and Oxidative Stress

	Marker	Cell Lines	Primary cells	3D Tissue	Ex vivo Tissue
Inflammation	Cytokines /Chemokines	X	X	X	X
	Nitric Oxide	?	X	X	X
	Prostaglandins	?	X	X	X
	Proteases			X	X
	Activated macrophages				X
	Neutrophil recruitment				
Oxidative stress	ROS	X	X	X	X
	Glutathione	X	X	X	X
	Lipid Peroxidation	X	X	X	X

Upcoming Technologies: E.g. Lung on a Chip

- **Wyss Institute** at Harvard has developed "breathing" human lung chip that **mimics airflow and bloodflow**
- Introduction of bacteria to lung cells triggers **white blood cell translocation** through porous membrane layer

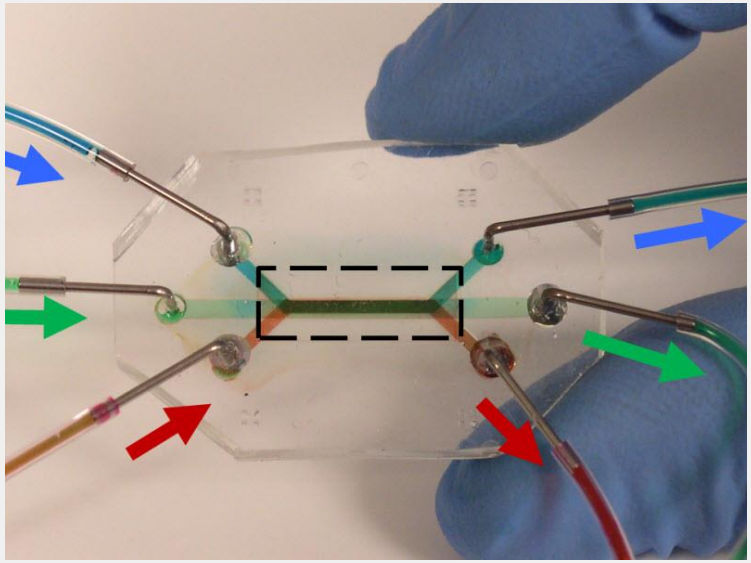
Researchers mimic pulmonary edema in lung-on-a-chip



The Wyss Institute's human breathing lung-on-a-chip, made using human lung and blood vessel cells, acts much like a lung in a human body. A vacuum re-creates the way the lungs physically expand and contract during breathing. In the current study, when researchers applied the cancer drug IL-2, fluid from the bottom of the chip entered the air channel on the top, and the blood clotted -- mimicking what happens when humans get pulmonary edema. Further, when they turned on the vacuum to simulate breathing, the fluid leakage was much worse -- adding new insight to what scientists understand about this life-threatening condition.

http://wyss.harvard.edu/viewpage/404/

- Researchers at **RTI International**, in collaboration with the **University of North Carolina at Chapel Hill**, have developed a new lung-on-chip microdevice
- The microdevice includes **multiple vertically stacked cellular layers** that mimic the structure of the airway tissue.



RTI's lung-on-a-chip emulates the multilayer airway tissue microarchitecture. In this picture, dyes are flown in the three vertically stacked compartments separated by transparent membranes (shown by the dotted rectangle).

http://www.rti.org/newsroom/news.cfm?obj=503D8210-E344-7120-553A0AD769F494B4

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Thank you!

Helpful references:

COPD

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- van den Berge, M. et al. (2011) Small Airway Disease in Asthma and COPD, Clinical Implications *Chest* 2011;139(2): 412–423

In Vitro Pulmonary Models

- BeruBe, K. et al. (2009) In Vitro Models of Inhalation Toxicity and Disease The Report of a FRAME Workshop *ATLA — Alternatives to Laboratory Animals*, Vol. 37, No. 1, 02.2009, p. 89-141.
- Jason Adamson, Linsey E Haswell, Gary Phillips and Marianna D Gaça (2011). In Vitro Models of Chronic Obstructive Pulmonary Disease (COPD), *Bronchitis*, Dr. Ignacio MartÃn-Loeches (Ed.), ISBN: 978-953-307-889-2, InTech, Available from: http://www.intechopen.com/books/bronchitis/in-vitro-models-of-chronicobstructive-pulmonary-disease-copd