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08

Session:
Biology-Inspired™ Tissue and Cellular Engineering

Cellular Interaction with Carbon Nanotubes in 3 D Tissue-engineered Airway Mucosa

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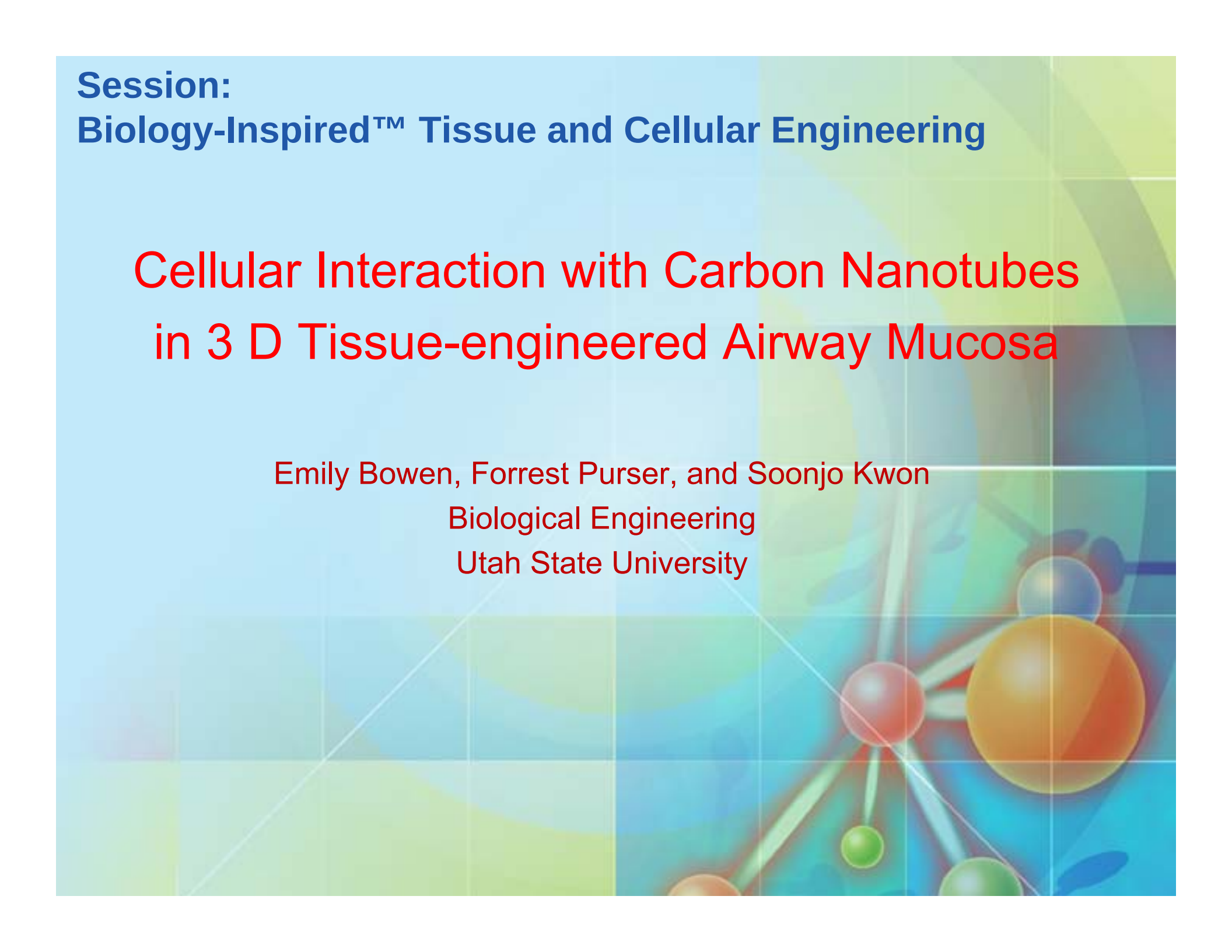
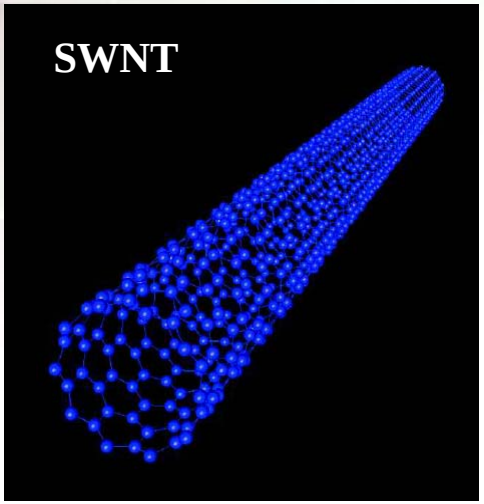
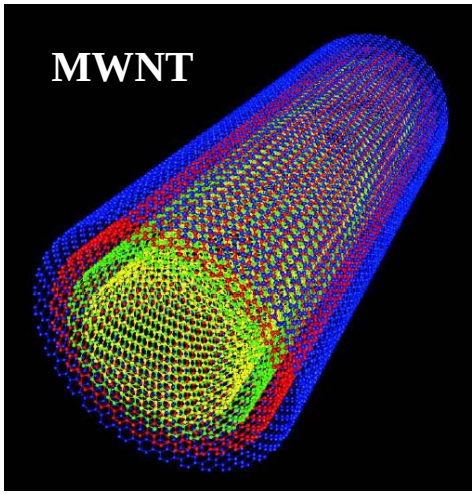


Carbon Nanotube Properties

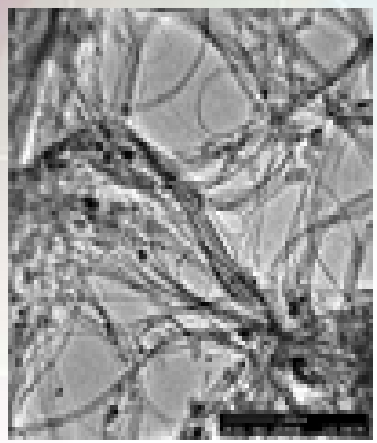
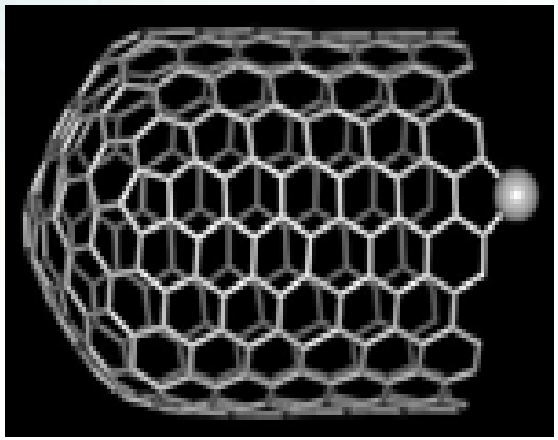
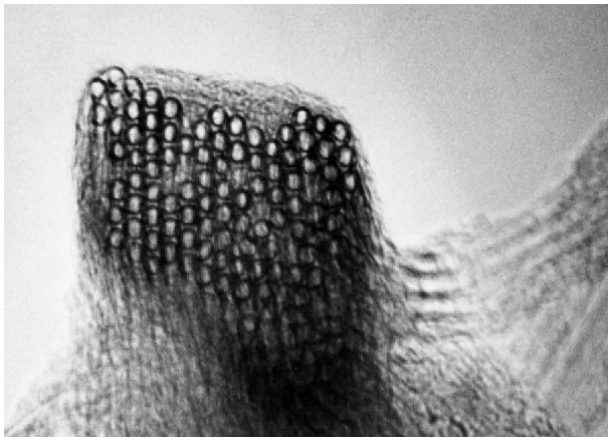
Carbon nanotubes (CNTs) are at the frontier of nanotechnology and destined to stimulate the next industrial revolution. The National Science Foundation forecasts that one trillion dollars' worth of nanotechnology-enabled products will be on the market by 2015.





Aspect ratio	~1000
Mechanical properties	Stiffness : 10-100 times higher than steel Elastic modulus : higher than 1 TPa (Dependent on their structure and diameter)
Electric-current-carrying capacity	1000 times higher than copper wires Resistance : 10^{-4} Ω/cm (at 300 K) Stability : 10^{13} A/cm (maximum)
Thermal conductivity	Twice as high as diamond 6000 W/m-K (at room temp.) (MWNT : 3000 W/m-K)
Thermal stability	2800°C in vacuum (800°C in atmosphere)

Micrographs of Carbon Nanotubes

In order to meet the growing demand of consumers, manufacturers are attempting to refine their fabrication process.

not only in the need to produce CNTs on a mass scale but also to protect their employees from the potential harmful effects of CNTs.

Motivation

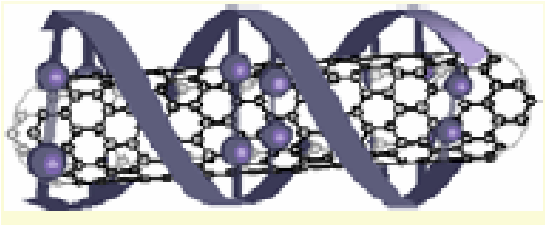
- The size of the CNTs makes them more readily become airborne and can therefore create the risk of being inhaled by a worker.
- Accidental exposure to CNTs might have adverse consequences to health, although the physicochemical determinants of these effects are not well characterized.
- Also, the available data for transport properties of carbon nanotube materials are very limited.

Despite the increasing list of potential CNT applications in the medical field, there is a major concern for potential inherent toxicity of CNTs and routes of exposure in biological systems.

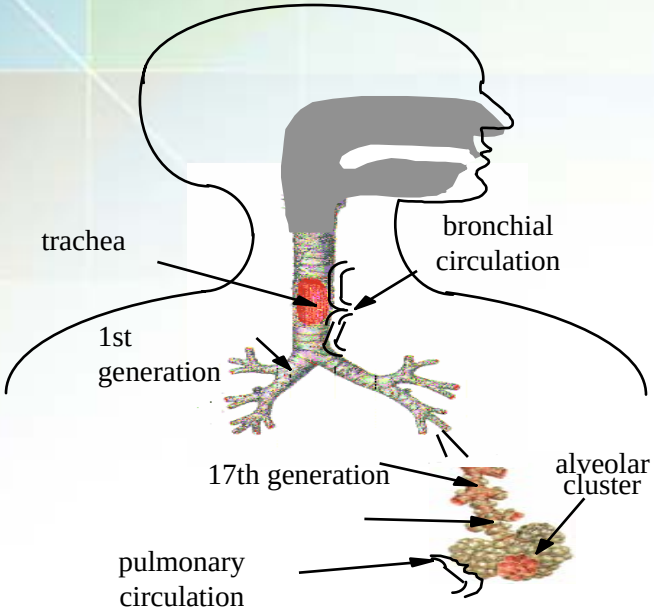
Human could be exposed to CNTs:

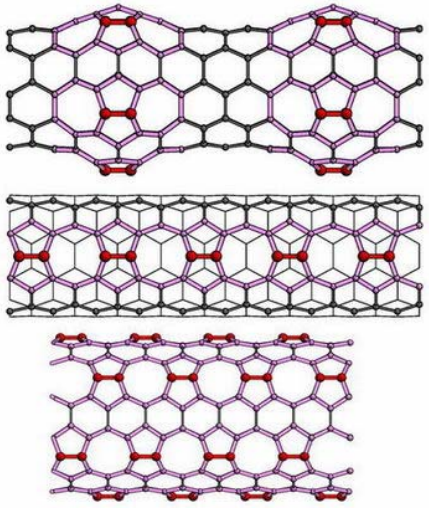
1. Accidental exposure, essentially to an aerosol in the context of CNT production and handling

2. Exposure as a result of CNT use for biomedical purposes.



Potential Health Risk of CNTs





- The small diameter/high curvature of individual fibers may lead to enhanced toxicity through mechanisms, such as reactive oxygen species generation in the lungs.

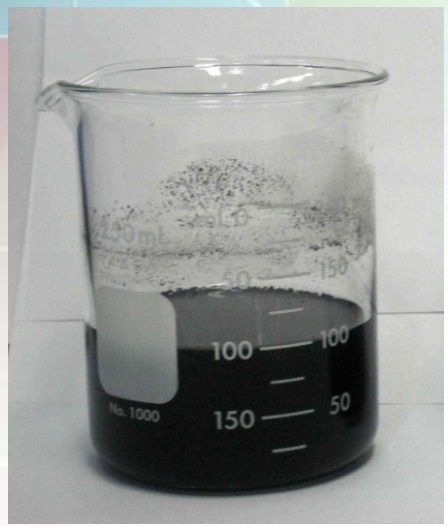
- Particle morphology may also interfere with lung clearance mechanisms, which again leads to the possibility of an enhanced toxicity compared to other forms of carbon.

OBJECTIVE

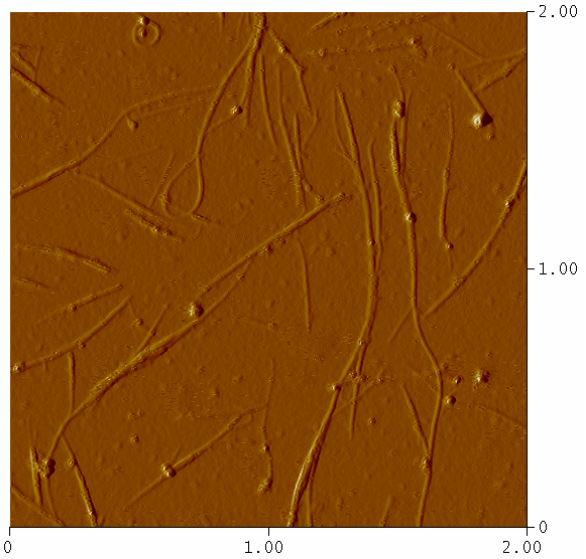
- To develop *viable alternatives to in vivo tests* to evaluate the toxicity of engineered CNTs

- To develop validated models capable of predicting the release, transport, transformation, accumulation, and uptake of CNTs in the human respiratory system.

Characterization of Aqueous SWCNT Suspension



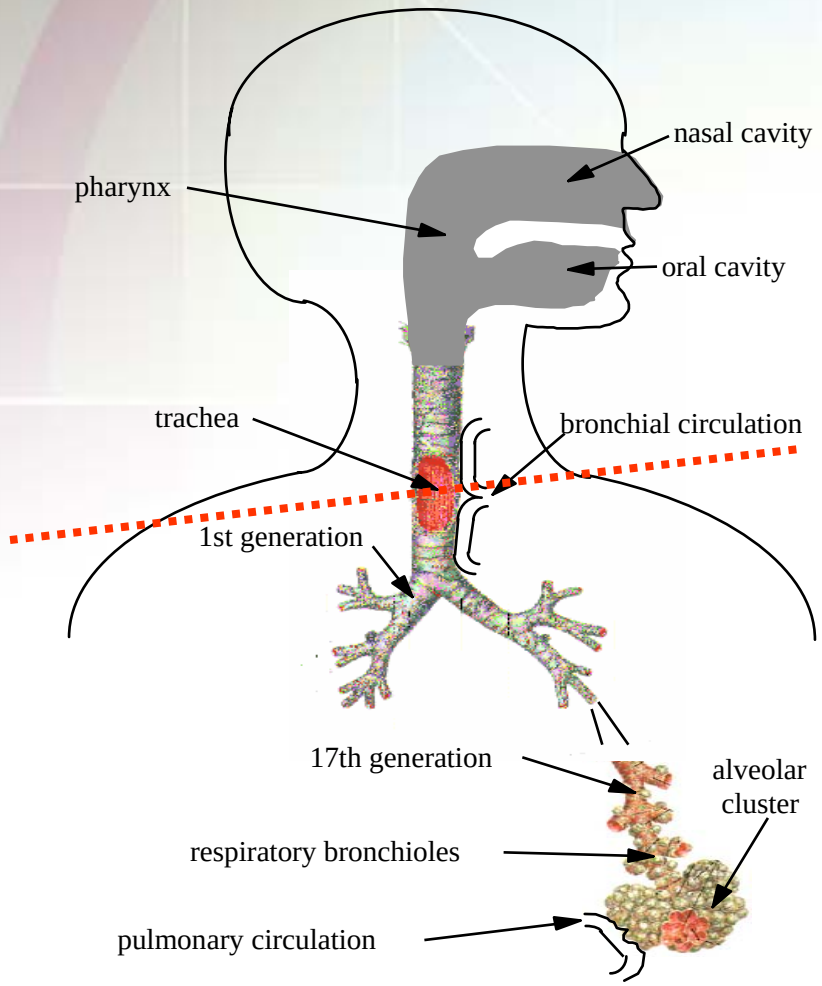
SWCNT suspension 12 hours after sonication

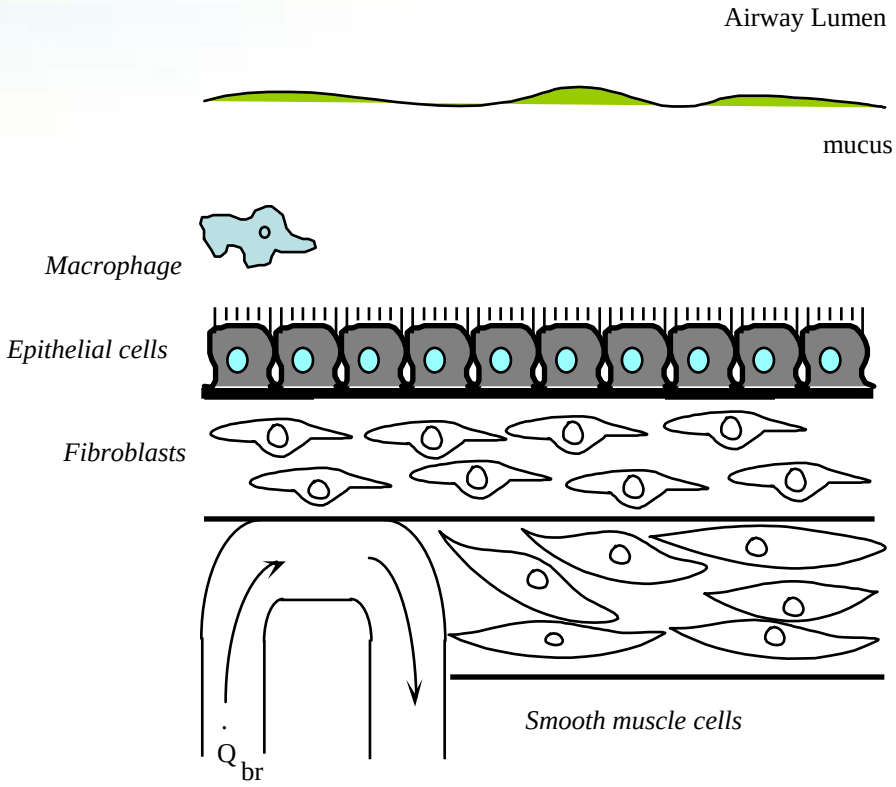


AFM image of diluted SWCNT dispersion

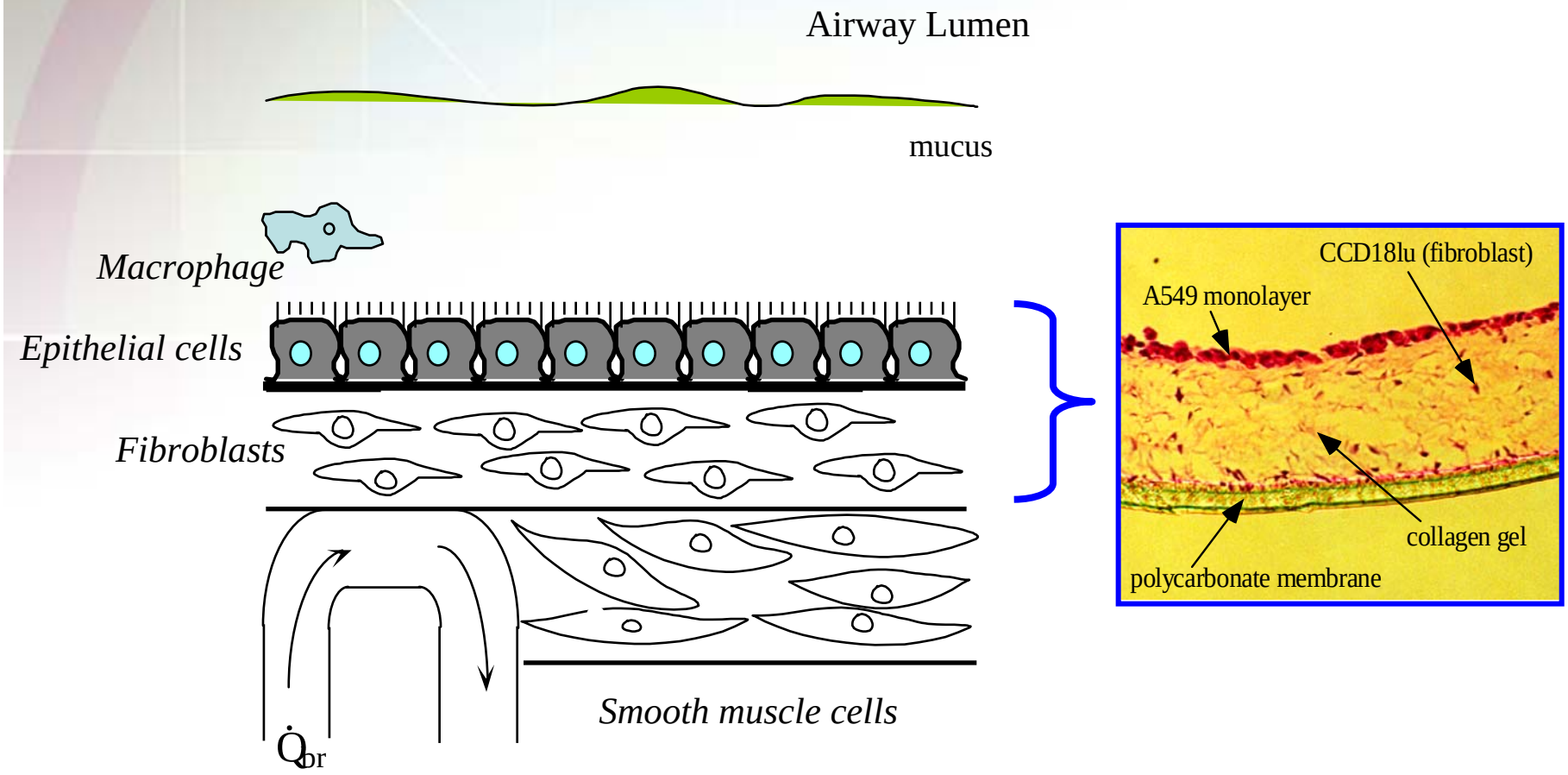
- SWCNT suspension
  - SWCNT + distilled water + surfactant (Triton X-100)
  - Processed using high-energy sonication
  - Suspension stayed stable for at least 2 months.
- Characterization
  - Atomic force microscopy
  - Mean CNT rope length ~ 500 nm
    Mean CNT rope diameter < 10 nm

Anatomy of Human Lung





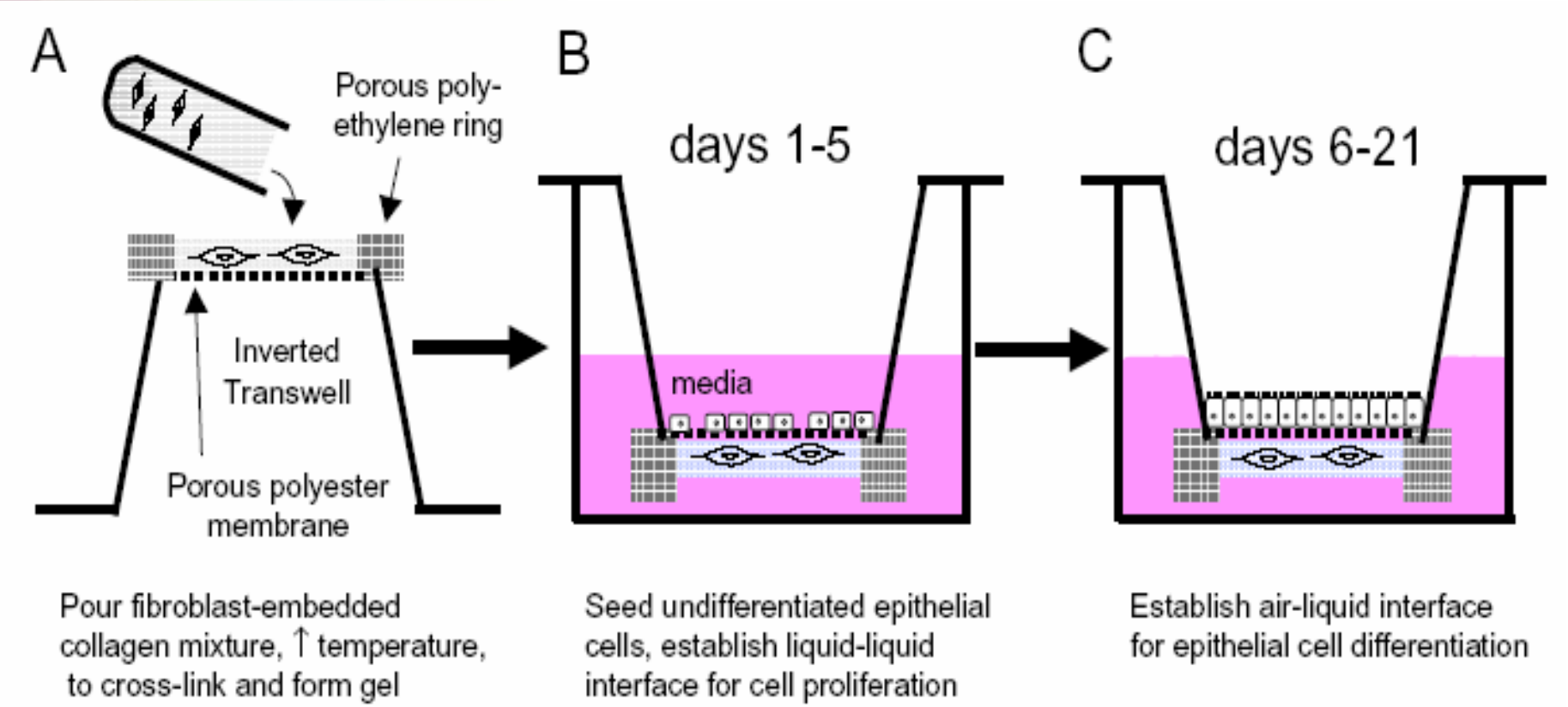
In-vitro Co-culture System



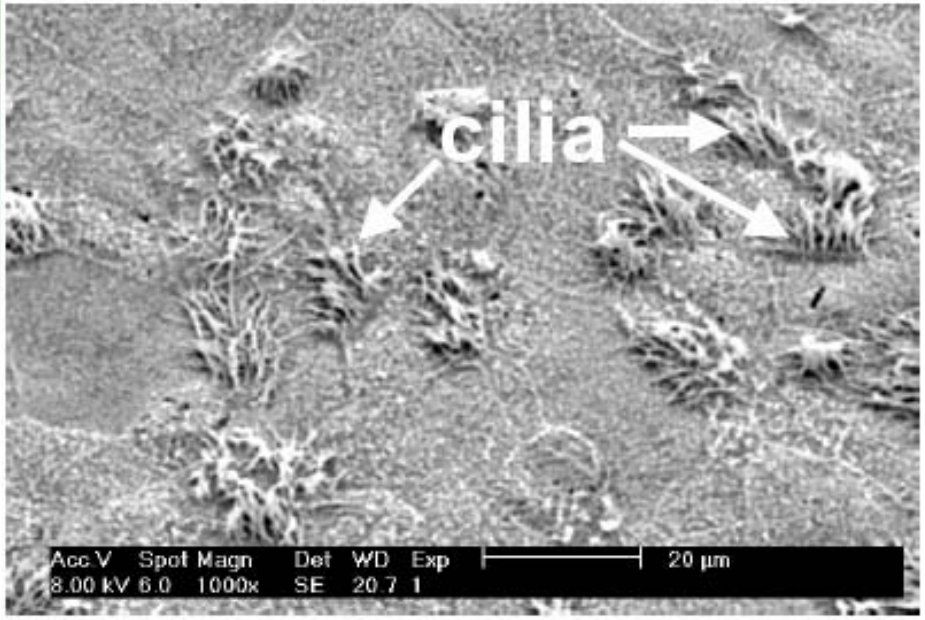
TISSUE-ENGINEERED BRONCHIAL MUCOSA

*The **normal human bronchial epithelial cells** are cultured as a monolayer over the thin polyester membrane and the **subepithelial human lung fibroblast-embedded collagen gel**.*

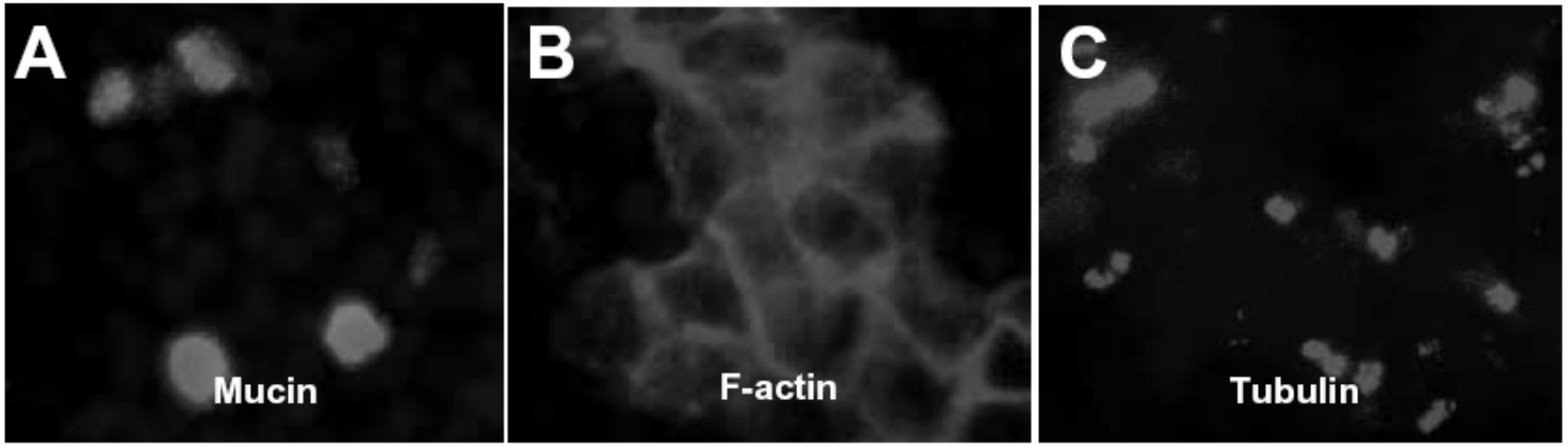
3 major steps and three weeks in culture.



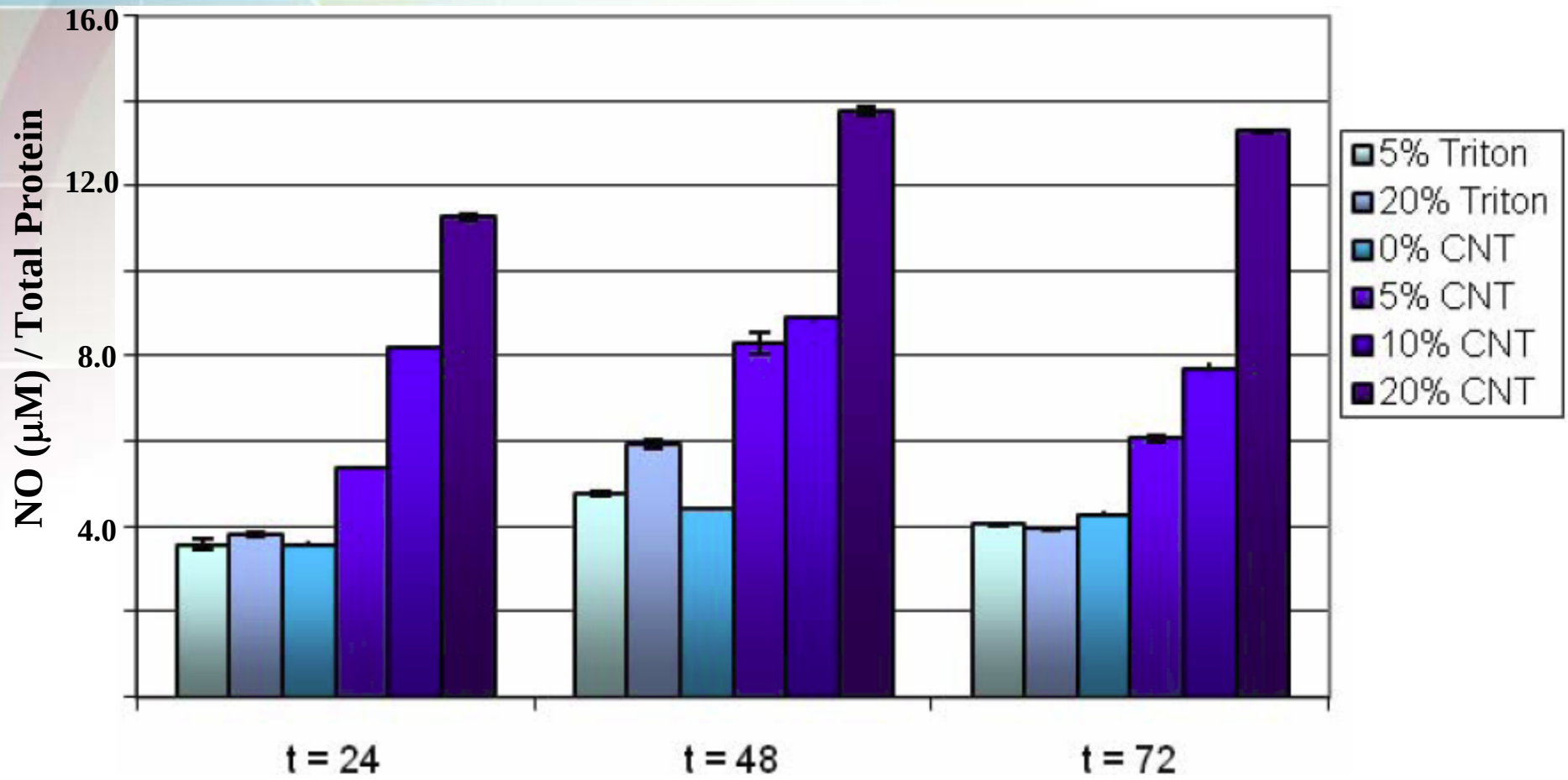
Physiological Characteristics of Airway Epithelial Cells



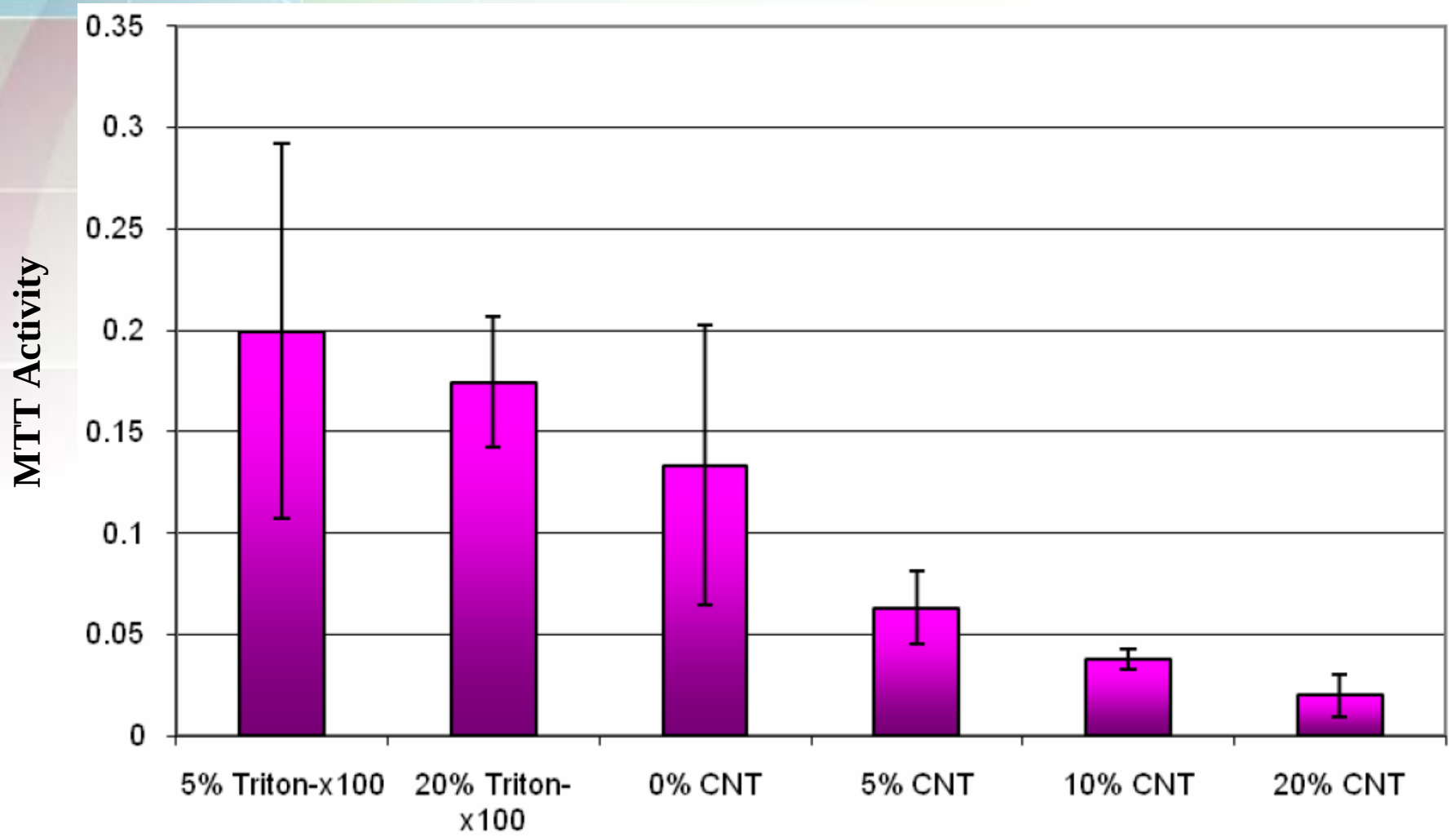
Cultured airway epithelium expresses three key phenotypic markers



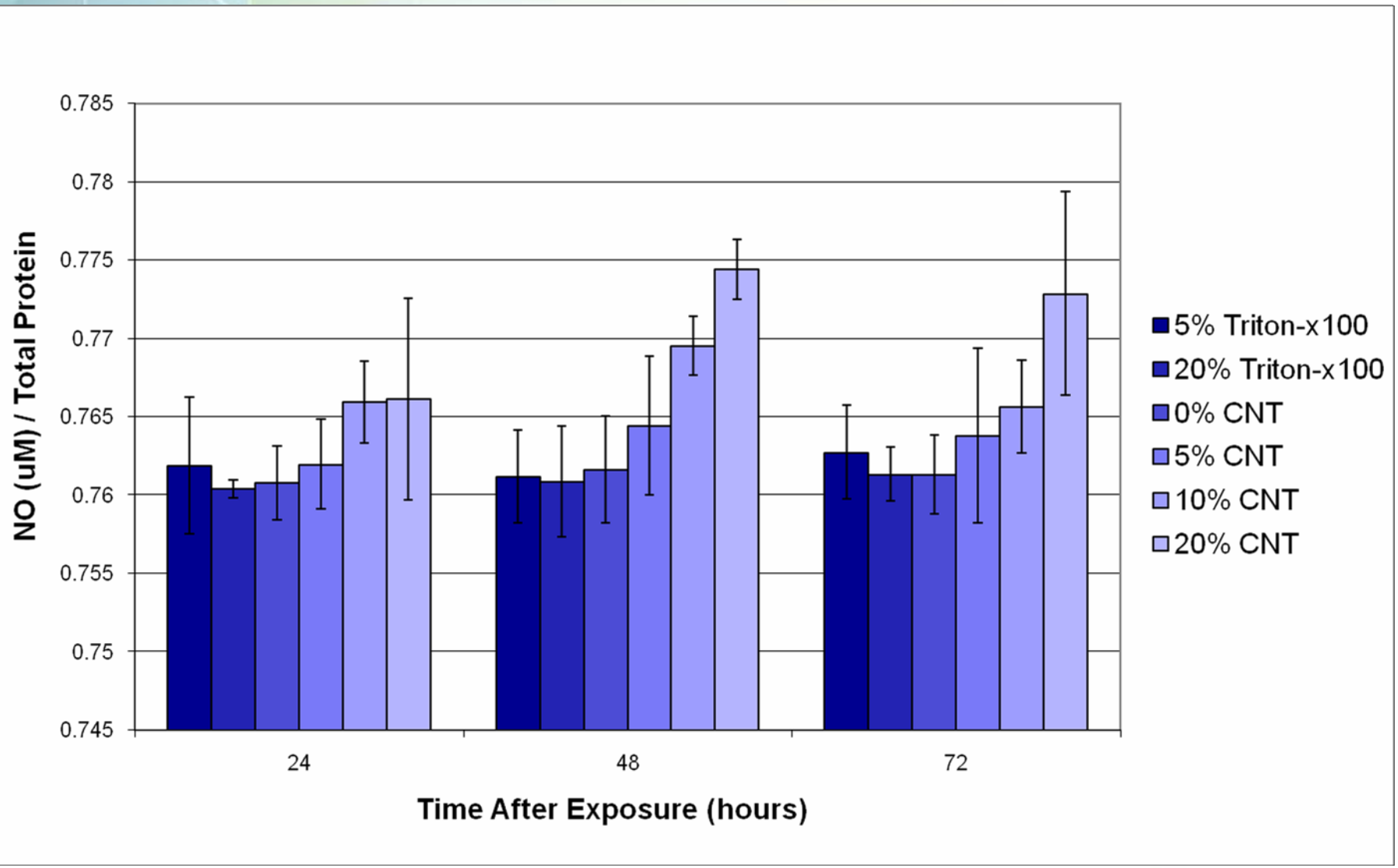
NO Production: Epithelial Cells



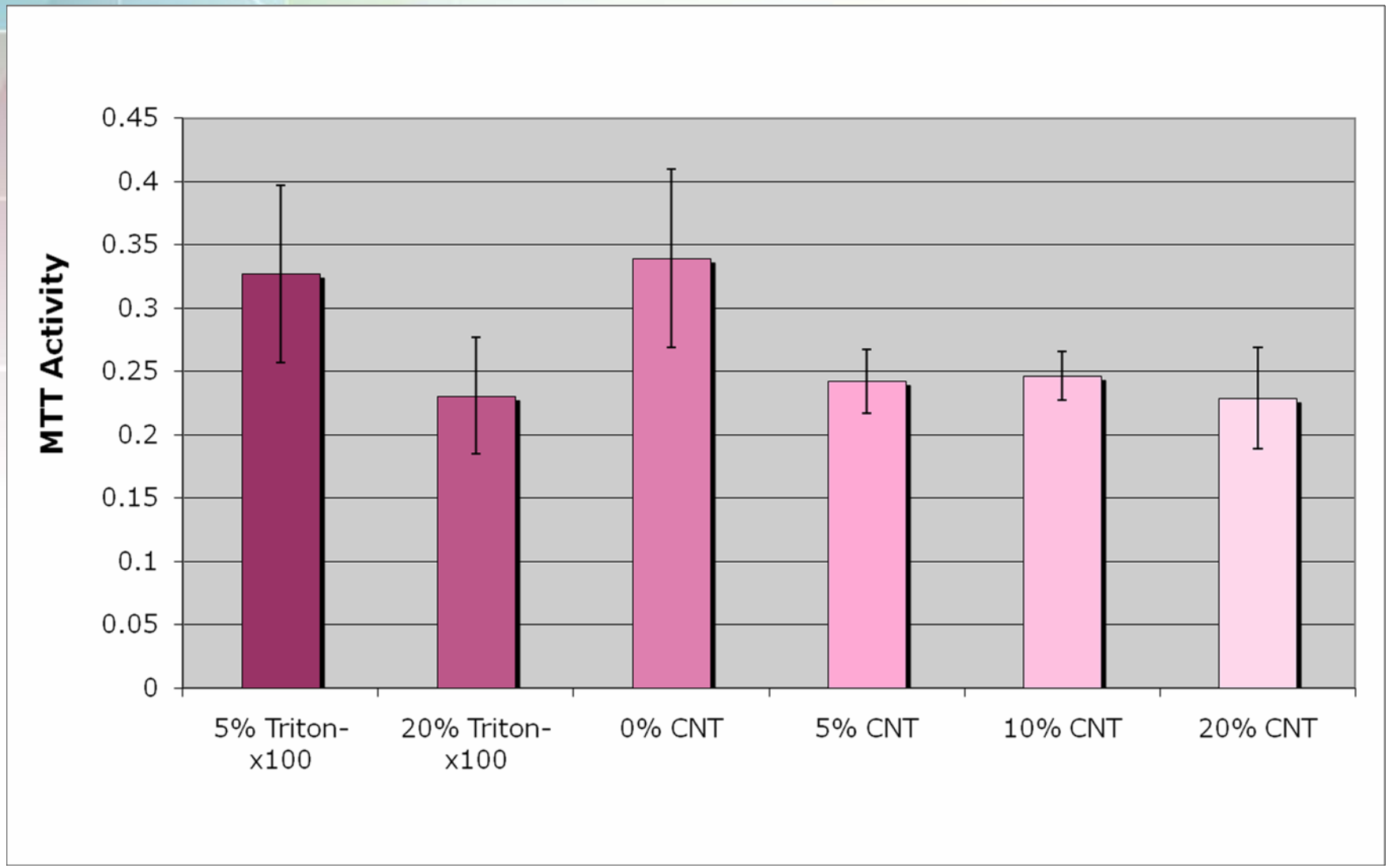
MTT : Epithelial Cells 48 hrs after exposure

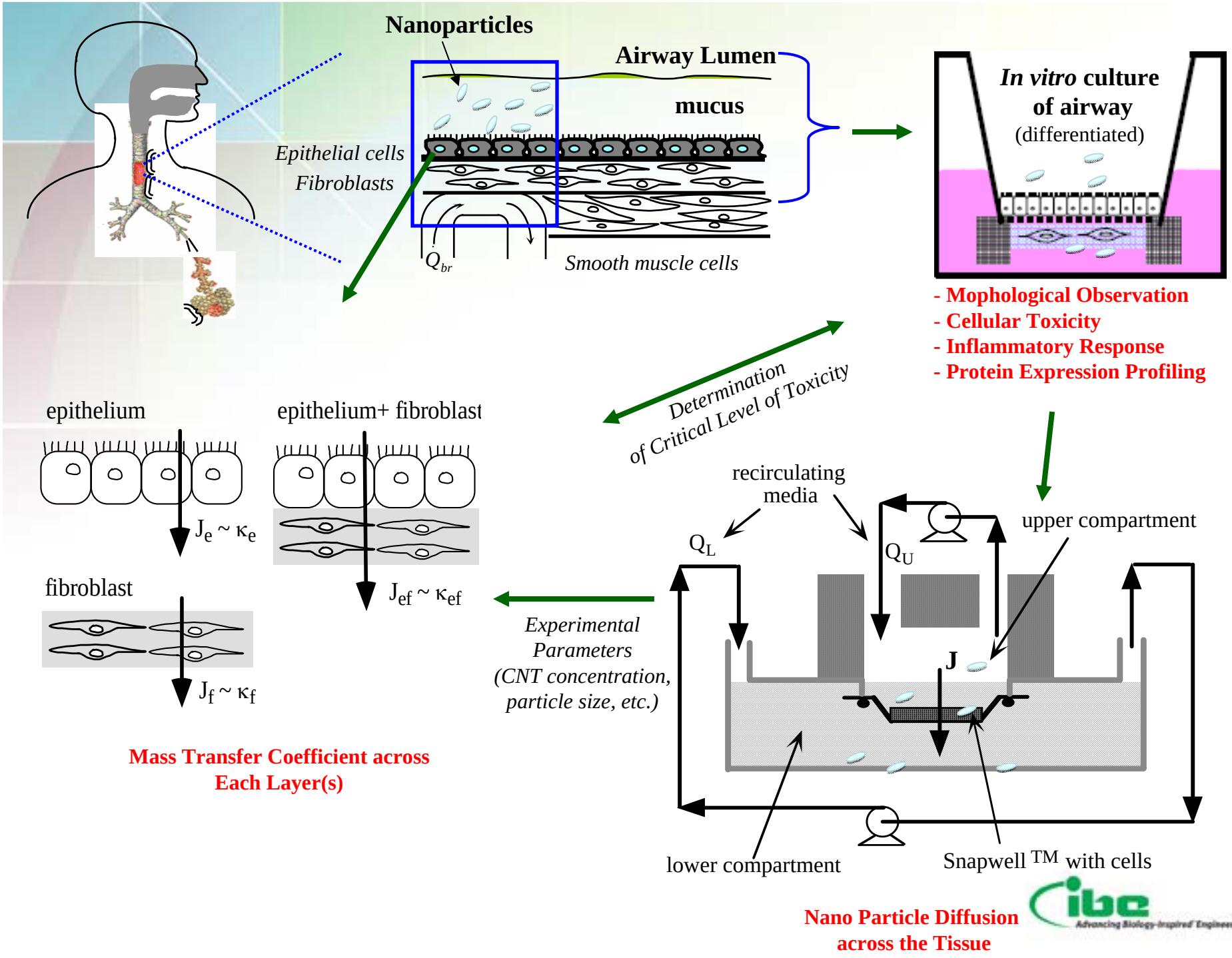


NO Production: Fibroblasts



MTT : Fibroblasts 48 hrs after exposure




Nanoparticles
Airway Lumen
mucus
Epithelial cells
Fibroblasts
Q_{br}
Smooth muscle cells
In vitro culture of airway
(differentiated)
- Mophological Observation
- Cellular Toxicity
- Inflammatory Response
- Protein Expression Profiling
Determination of Critical Level of Toxicity
epithelium
epithelium+ fibroblast
$J_e \sim \kappa_e$
$J_{ef} \sim \kappa_{ef}$
fibroblast
$J_f \sim \kappa_f$
Mass Transfer Coefficient across Each Layer(s)
Experimental Parameters (CNT concentration, particle size, etc.)
recirculating media
Q_L
Q_U
upper compartment
J
lower compartment
Snapwell ™ with cells
Nano Particle Diffusion across the Tissue
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Conclusion

- Correct anatomical arrangement, yet allows **clean access to both cell types for Proteomics Analysis**.
- NO production increased with increasing CNT concentrations.
- The MTT assay also demonstrated a decrease in cellular metabolic activity when exposed to CNT materials.
- *Cellular toxicity*, *Physiological Phenotypes*, and *Inflammatory Protein Expression* are being measured from normal human bronchial epithelial cells, either in the presence or absence of normal human lung fibroblasts.
- *Mass transfer coefficient* and *net fluxes* of each CNT will be measured through: 1) each individual cell monolayer, and 2) co-culture of epithelial cells and fibroblasts.

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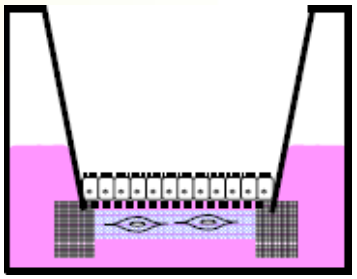
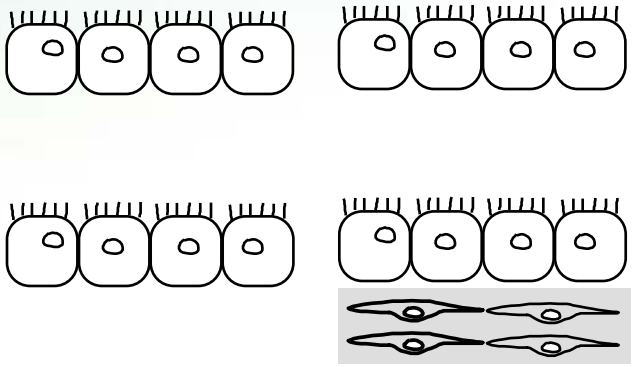
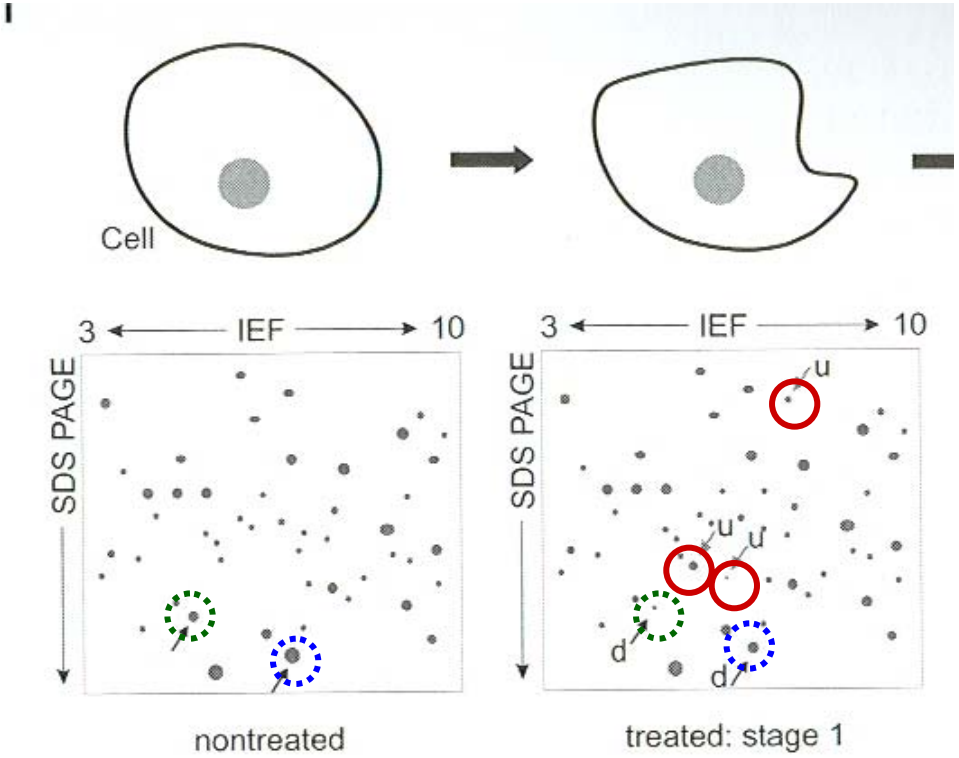
ongoing work

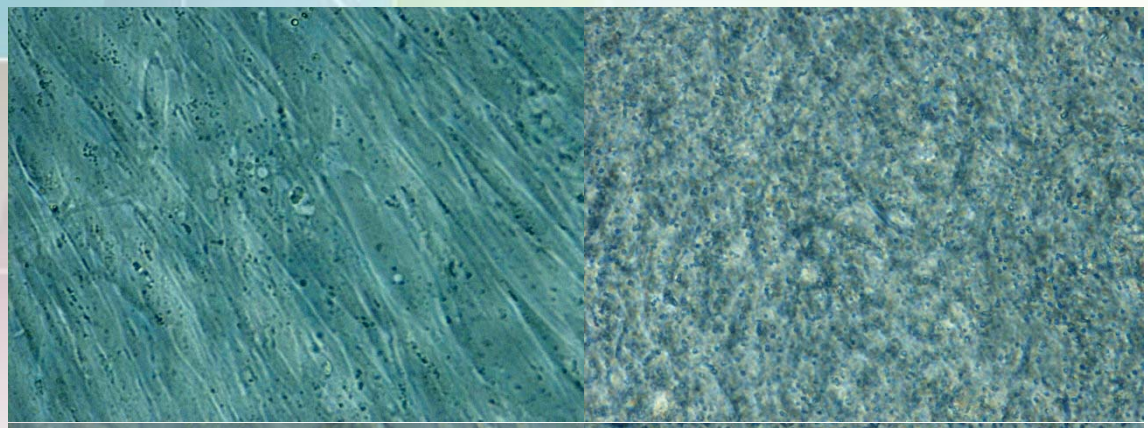
Important Features of Our Co-culture System



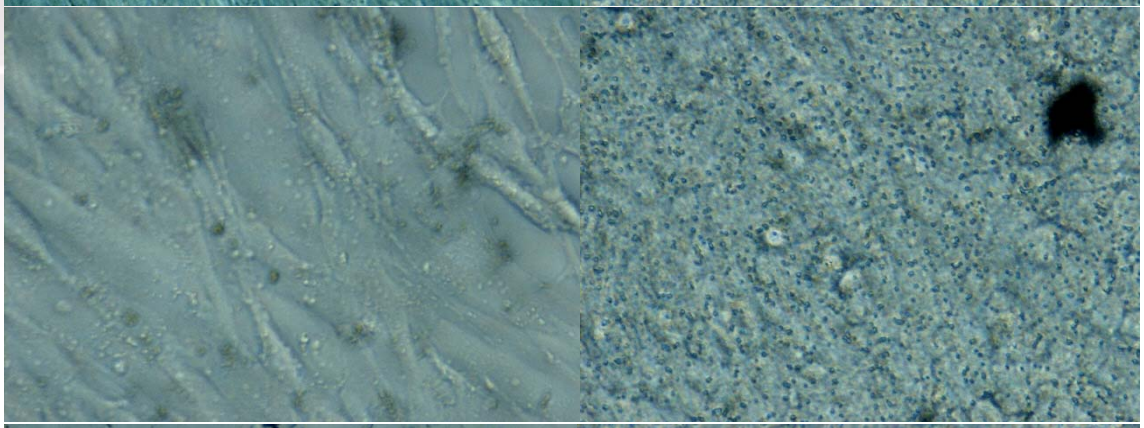




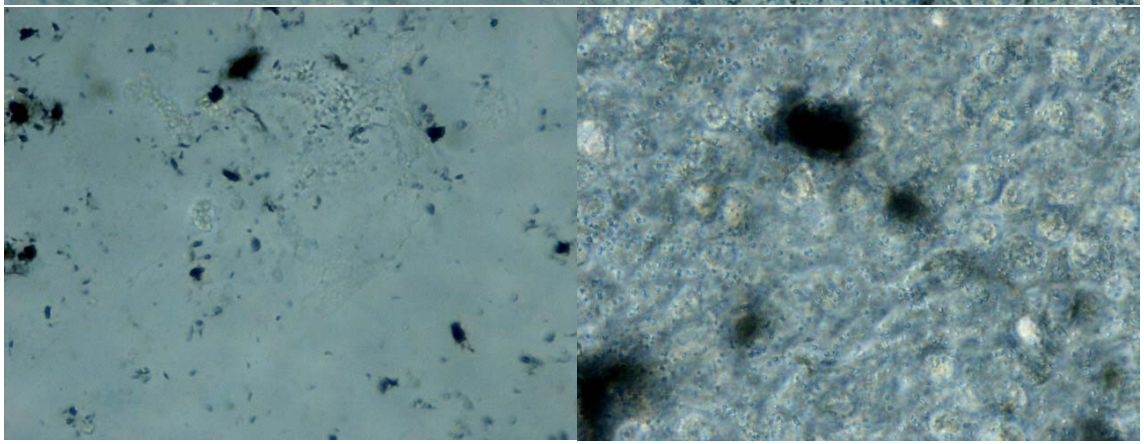
FIBROBLAST CELLS A549 CELLS



Control – no CNT or Triton at 48 hours

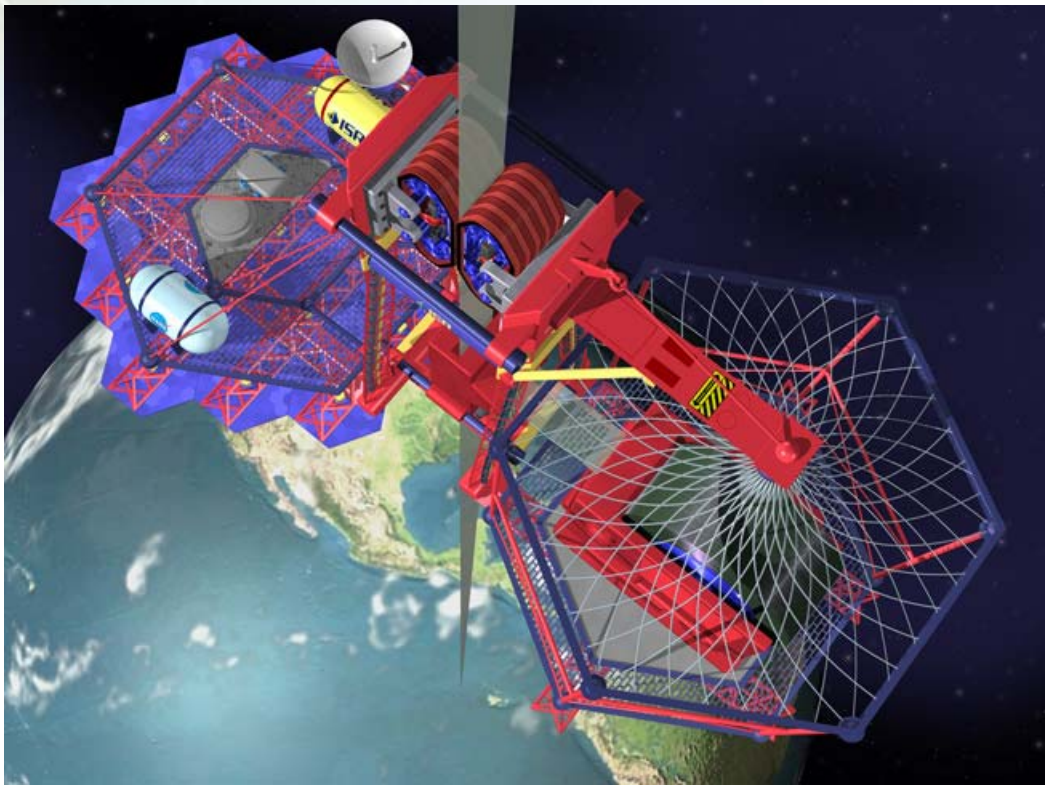


5% CNT- exposed to 5% CNT for 48 hours



20% CNT- exposed to 20% CNT for 48 hours

“Space Elevator”



“*A ribbon 62,000 miles (100,000 kilometers) long made of carbon nanotubes would be some three feet (less than a meter) wide and thinner than a newspaper page. But that ribbon would be hundreds of times sturdier than steel and one-fifth the weight.*“

Article by Leonard Davis, www.space.com, 9/17/2003.