Characterization and use of organotypic lung model to assess the pulmonary fibrosis of nanomaterials

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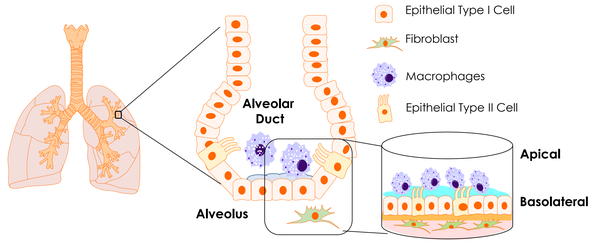
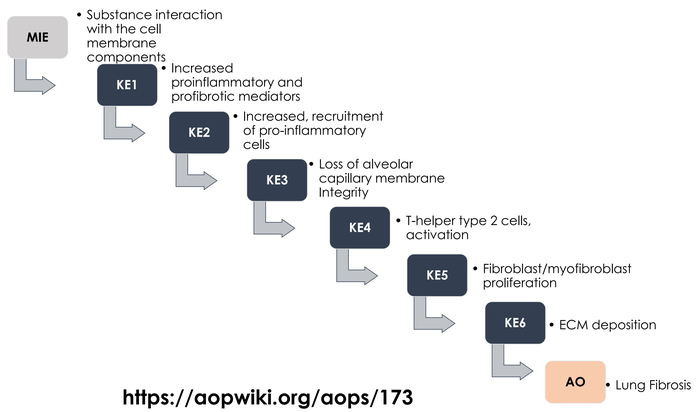
Abstract

Inhalation is one of the major routes by which exposure to nanomaterials (such as multi-walled carbon nanotubes (MWCNTs)) may occur and lead to adverse effects in humans, such as pulmonary fibrosis. Presented here is a reconstructed primary human alveolar cell-based model (EpiAlveolar™, MatTek Corp.) that can be used to assess the potential of test materials to cause pulmonary fibrosis. EpiAlveolar™ —comprised of alveolar epithelial cells, pulmonary endothelial cells, and fibroblasts—was developed to mimic the *in vivo* human alveolar microenvironment. Characterization of the model shows presence of alveolar epithelial Type I (ATI) and Type II (ATII) cells, expression of pro-surfactant C, and formation of a tight epithelial barrier. When challenged with transforming growth factor β (TGF-β)—a known positive control for pulmonary fibrosis—the EpiAlveolar™ model demonstrated gradual signs of fibrosis over a period of 21 days such as a significant decrease in barrier integrity (transepithelial electrical resistance (TEER)), increase in extra-cellular matrix proteins (fibronectin and collagen 1a1), and thickening of tissues (hematoxylin and eosin staining). The aforementioned fibrotic biomarkers were also expressed after repeated sub-chronic exposures (1 – 30 µg/cm² for up to 21 days) to two types of MWCNTs (Mitsui-7 and Nanocyl) using an air-liquid interface exposure device (VITROCELL® Cloud). The EpiAlveolar™ model has been described in Barosova et al. 2020 and has shown value in measuring several key events along the proposed adverse outcome pathway for pulmonary fibrosis (https://aopwiki.org/aops/173). This system can be used in a mechanism-based in vitro testing strategy using human-relevant methods to predict pulmonary toxicity and to enable effective risk assessment of substances including MWCNTs.

Mechanism and conceptual model

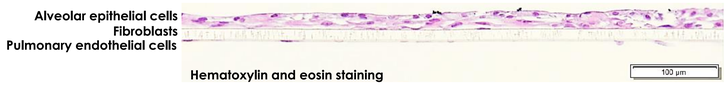


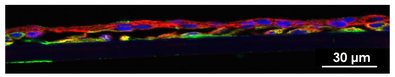


https://aopwiki.org/aops/173

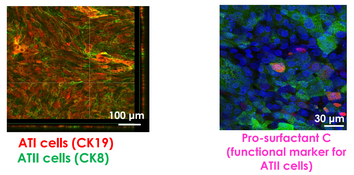
Model development and characterization

EpiAlveolar tissues are 2-4 cell layers thick

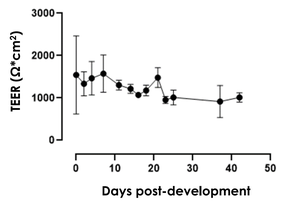


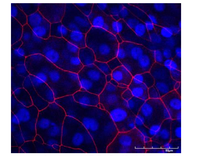


EpiAlveolar tissues have alveolar type I and II cells



EpiAlveolar tissues form tight epithelial barrier



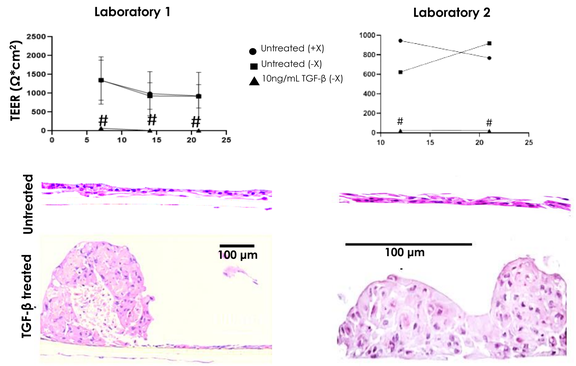


EpiAlveolar™ Tissues:

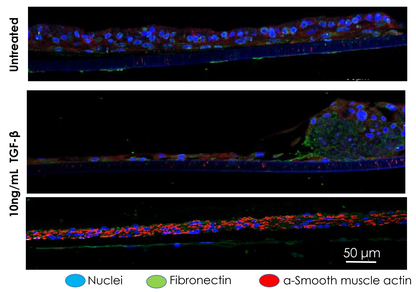
- Co-culture of primary human alveolar epithelial cells, pulmonary endothelial cells, and human fibroblasts, with or without macrophages
- 2-4 cell layers thick on the apical surface with a thin monolayer of endothelial cells on the basal surface
- Alveolar type I and II cells are present
- Demonstrates tight tissue barrier (>1000 Ω*cm²)
- Cultured on Transwells
- Commercially available

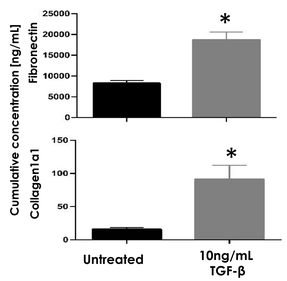
Assessment of Pro-fibrotic Response

TGF-β induces (pro-)fibrotic response in EpiAlveolar™ tissues

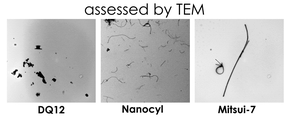


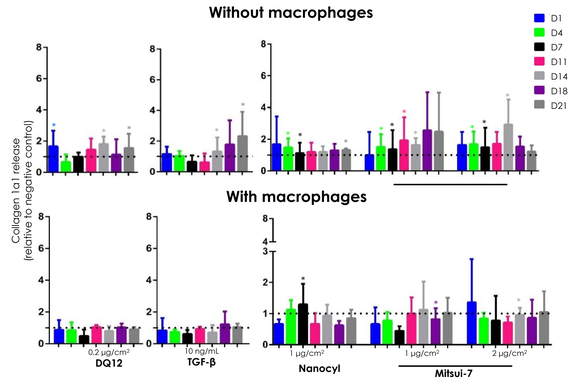
TGF-β induces increase in extracellular matrix proteins





Nanomaterial-induced pro-fibrotic response seems to be depended on macrophages





Observations and next steps:

- After TGF-β treatment, EpiAlveolar tissues began to contract away from the Transwell insert and round up. This contraction resulted in reduced TEER and, subsequently, complete loss (statistically significant decrease; ~ 10 Ω*cm²) of TEER at D21. This finding was observed in two laboratories
- TGF-β treatment of EpiAlveolar tissues increased release of extracellular matrix proteins, such as fibronectin and collagen (as measured by enzyme-linked immunosorbent assay (ELISA))
- Immunofluorescence staining for fibronectin was increased in the EpiAlveolar tissues treated with TGF-β for D21 compared to untreated tissues
- Collagen1a1 increase after treatment with nanomaterials seems to be dependent on the presence of macrophages
- Nanomaterial testing with EpiAlveolar tissues will continue under the Horizon 2020 project PATROLS (Physiologically Anchored Tools for Realistic nanOmateriaL hazard aSsessment)