Development of a Cell-Based Approach to Assess Pulmonary Fibrosis Following Exposure to Nanomaterials

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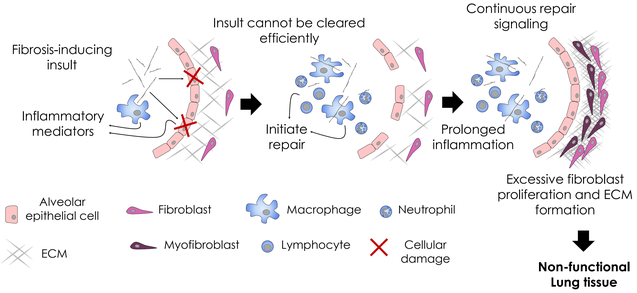
INTRODUCTION

Inhalation exposure to nanomaterials (NM), such as multi-walled carbon nanotubes (MWCNTs), has been linked to adverse health effects *in vivo*, such as pulmonary fibrosis. Current regulatory testing requirements for substances that have the potential for inhalation exposure include a 90-day rodent inhalation test. Due to ethical, monetary, and scientific concerns associated with the rodent inhalation test, non-animal approaches are currently being developed to assess human health hazard. The goal of this work is to develop an *in vitro* testing strategy using human-relevant methods to predict pulmonary toxicity and to enable effective risk assessment. Presented here is a model assessment of immortalized human cell line cultures and primary cell (MatTek EpiAlveolar™)cultures following exposure to known pro-fibrotic stimuli to assess if the *in vitro* system can predict the human outcome.

MECHANISM OF PULMONARY FIBROSIS



Adverse outcome pathways

Molecular Initiating Event → Cellular Response → Organ Response → Organism Response

- **MIE** • Substance interaction with the cell membrane components
- **KE1** • Increased proinflammatory and profibrotic mediators
- **KE2** • Increased, recruitment of pro-inflammatory cells
- **KE3** • Loss of alveolar capillary membrane Integrity
- **KE4** • T-helper type 2 cells, activation
- **KE5** • Fibroblast/myofibroblast proliferation
- **KE6** • ECM deposition
- **AO** • Lung Fibrosis

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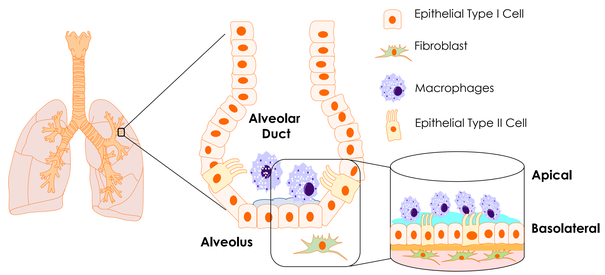
MODEL DEVELOPMENT

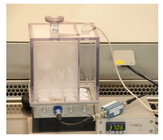
CONCEPTUAL MODEL TO PREDICT PULMONARY FIBROSIS



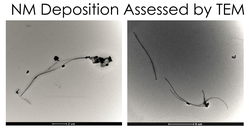
NM CHARACTERIZATION, EXPOSURE, AND DOSIMETRY

Two types of MWCNTs were tested: Nanocyl 7000s (JRC NM 400) and Mitsui-7s.

VITROCELL® Cloud system: NM Generation and Exposure System



NM Deposition Assessed by TEM

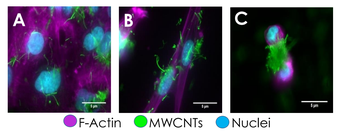


NM Deposition Assessed by QCM (µg/cm²)

Test	Deposited Mass/Nebulization
BSA	0.2
Nanocyl	0.5
Min-U-Sil	0.7
Mitsui-7	1
Mitsui-7	2

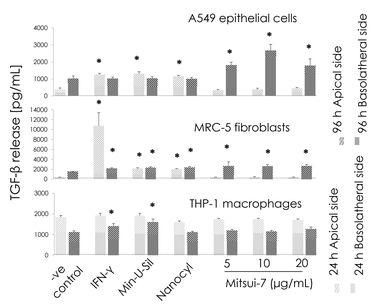
Next step
To use a dry aerosol generator (VITROCELL Powder Chamber) to aerosolize NMs and expose the cells

CELL LINES: MONO-CULTURES



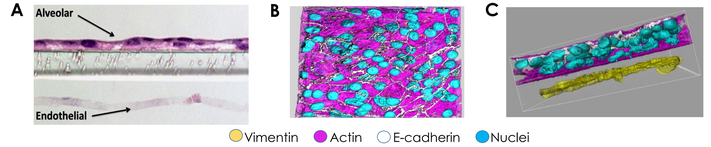
Dark field images of (A) A549, (B) MRC-5, and (C) THP-1 cell lines exposed to Mitsui-7 MWCNTs suspension at 10 µg/mL after 24 h.

Observation
A notable increase in TGF-β release was observed in MRC-5 cells and A549 cells following Mitsui-7 treatment (5, 10, or 20 µg/mL) compared to the negative controls.

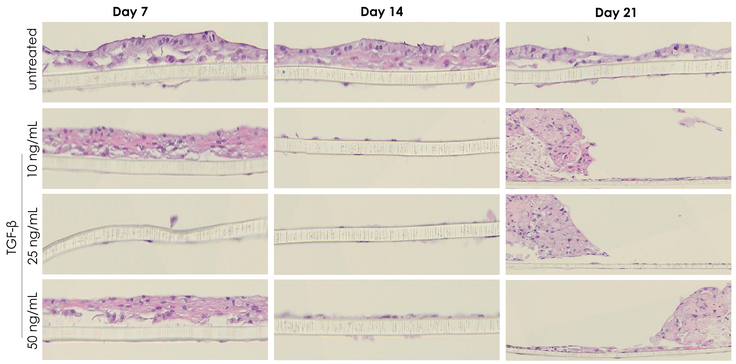


The three cell lines were cultured and exposed in suspension to MWCNTs (Mitsui-7s and Nanocyl dispersed in water with 0.1% BSA) at different concentrations. After exposure for 24 or 96 h, the supernatant was collected and tested for different biomarkers of pro-inflammation and pro-fibrosis. Interferon-γ and Min-U-Sil were used as positive controls, and cells cultured in media with BSA were used as negative (-ve) controls. TGF-β release was assessed relative to negative controls. The graphs represent n=3; IFNγ=1µg/mL; error bars = standard error of mean; * = significant increase ($p < 0.05$).

PRIMARY CO-CULTURE: EPIALVEOLAR™

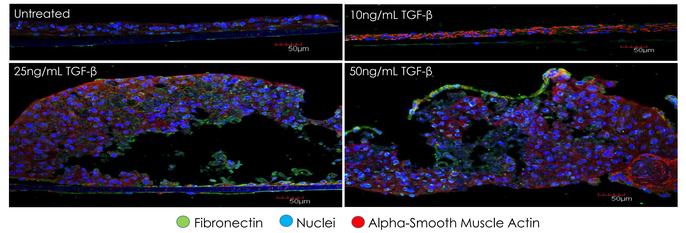


A hematoxylin and eosin stained section of prototype(A) and LSM images (B and C) of EpiAlveolar™ model is shown. Cell morphology was assessed using immunostaining in B and C.



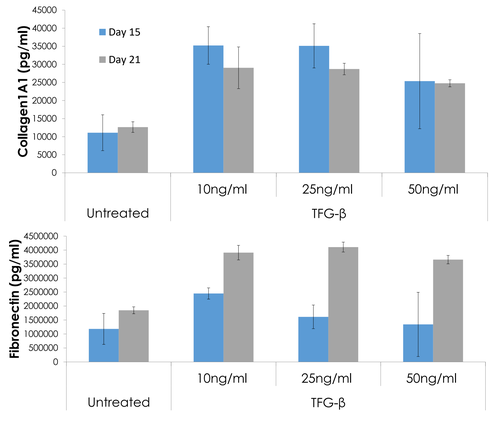
EpiAlveolar™ model cultured in medium was treated with TGF-β at different concentrations (10, 25, or 50 ng/mL) as a positive control. Hematoxylin and eosin staining was performed after 7, 14, and 21 days to assess tissue morphology.

Observation
Tissue contraction and a significant increase in cell number was observed at days 14 and 21 for all tested concentrations of TGF-β. Tissue contraction lead to detachment and loss of cells from the membrane as observed in images for day 14.



EpiAlveolar™ model was treated with TGF-β at different concentrations (10, 25, or 50 ng/mL). Immunostaining was used to assess the expression of alpha-smooth muscle actin and fibronectin. Shown here are representative images for day 21 after treatment.

Observation
A significant increase in fibronectin and alpha-smooth muscle actin was observed at all tested concentrations of TGF-β.



EpiAlveolar™ model was treated with TGF-β at different concentrations (10, 25, or 50 ng/mL). Supernatant was collected after day 15 and 21. Levels of pro-fibrotic biomarkers were assessed relative to negative controls using Bio-Plex® Multiplex Immunoassay System. The graphs represent n=1 with 3 replicates per treatment.

Observation
A trend towards an increase in collagen 1A1 and fibronectin was observed after day 15 and 21 of treatment with TGF-β

Next step
Repeat the treatment conditions at additional test concentrations and time points.

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