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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 EpiAlveolarTM)cultures following exposure to known pro-fibrotic stimuli to assess if the in vitro system can predict the human outcome.



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

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CONCEPTUAL MODEL TO PREDICT PULMONARY FIBROSIS Epithelial Type I Cell Fibroblast Macrophages Alveolar Epithelial Type II Cell Alveolus

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



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

F-Actin OWCNTs Ouclei Dark field images of (A) A549, (B) MRC-5, and (C) THP-1 cell lines exposed to Mitsui-7 MWCNTs suspension at 10

 μ g/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; IFNy=1 μ g/mL; error bars = standard error of mean; * = significant increase (p < 0.05).

MODEL DEVELOPMENT



NM Deposition Assessed by QCM (µg/cm²)











E-cadherin Nuclei

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.

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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-B. 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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