PATROLS

Advanced Tools for NanoSafety Testing

Advanced lung models with (patho)physiological relevance to assess long-term effects of engineered nanomaterials

Background

PATROLS focused on improving the (patho)physiological relevance of in vitro lung models by the introduction of fluid-flow and mechanical motion and provision of models of common human lung disease to understand pulmonary sensitivity to long-term and repeated engineered nanomaterial (ENM) exposure.

(Long-term) repeated ENM exposures to predict (pro-)inflammatory and (pro-)fibroticendpoints in lungmodels

- Lung co-cultures of epithelial cell lines and macrophages (primary cells / cell lines) have been designed to investigate the (pro-)inflammatory endpoints upon repeated ENM exposures [1, 2].
- A co-culture model composed of human cell lines, i.e. alveolar epithelial cells, fibroblasts, and macrophages, can be used to assess the onset of (pro-)inflammatory response upon repeated exposures (up to 3 days) to ENM [3].
- The EpiAlveolarTM model (MatTek Corporation) made of primary cells can predict the (pro-)fibrotic

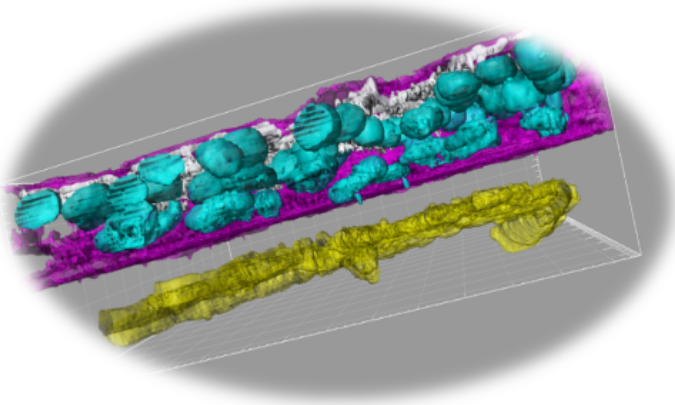
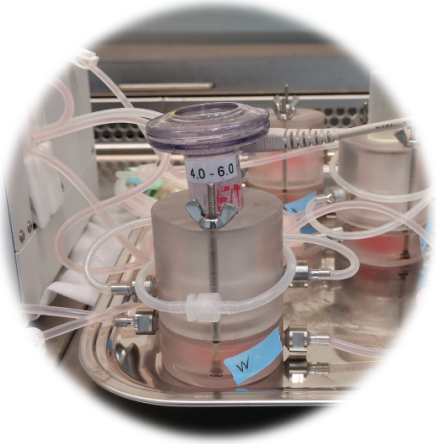


Figure 1.3D reconstruction of a confocal data set representing human epithelial type I cells and fibroblasts on the upper side of a transwell insert and endothelial cells on the lower side.



Bioreactor design to mimic breathing motions

- In order to improve the physiological relevance of the lung model, a bioreactor including fluid-flow and mimicking breathing movements, was designed.
- The device consists of a bioreactor chamber with a moving membrane placed at air-liquid interface and an aerosol generator can be connected to create an aerosol of ENM.

Figure 2. The assembled bioreactor connected to a perfusion system and an aerosol generator.

[1] Barosova, et al. Multicellular Human Alveolar Model Composed of Epithelial Cells and Primary Immune Cells for Hazard Assessment. J. Vis. Exp., e61090, 2020. [2] Braakhuis et al. An Air-liquid Interface Bronchial Epithelial Model for Realistic, Repeated Inhalation Exposure to Airborne Particles for Toxicity Testing. J. Vis. Exp., e61210, 2020. [3] Barosova et al. An In Vitro Lung System to Assess the Proinflammatory Hazard of Carbon Nanotube Aerosols. Int J Mol Sci, 21, 2020. [4] Barosova et al. Use of EpiAlveolar Lung Model to Predict Fibrotic Potential of Multiwalled Carbon Nanotubes. ACS Nano, 2020.

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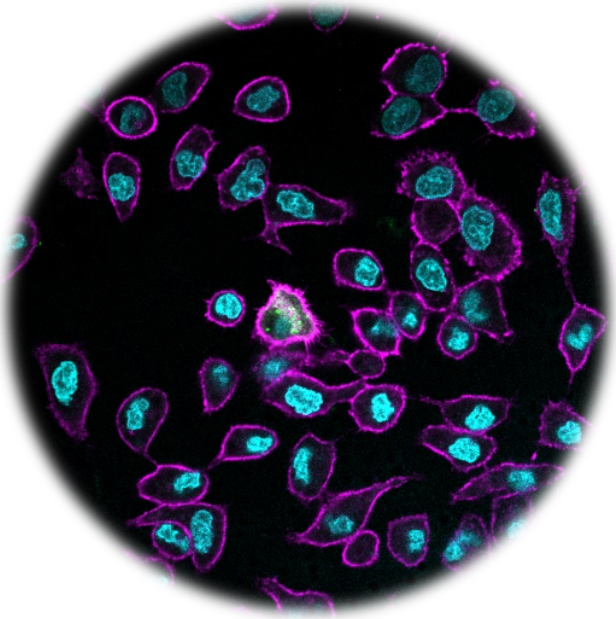

Figure 3. Morphology of human alveolar epithelial cells and human monocyte-derived macrophage visualized via confocal laser scanning microscopy.

Inflamed lung model

- An inflamed model of human alveolar epithelium, composed of human primary macrophages, along with an alveolar epithelial type II cell line, and human monocyte-derived dendritic cells was optimized to assess induction of a (pro-)inflammatory response from the system.
- The cells were stimulated with lipopolysaccharide (LPS) from the basal side, i.e. simulating a systemic alveolar inflammation providing a physiologically relevant model for nanomaterial hazard assessment associated with diseased individuals, i.e., suffering acute or chronic lung inflammation [5].

Comparison of indirect and direct ENM exposures

- The pro-fibrotic effects of ENM on lung fibroblasts can be assessed to mimic the pulmonary environment of this cell type during inhalation exposure
- Pro-fibrotic responses to ENM after acute direct exposure has been compared to chronic exposure in human lung fibroblasts.

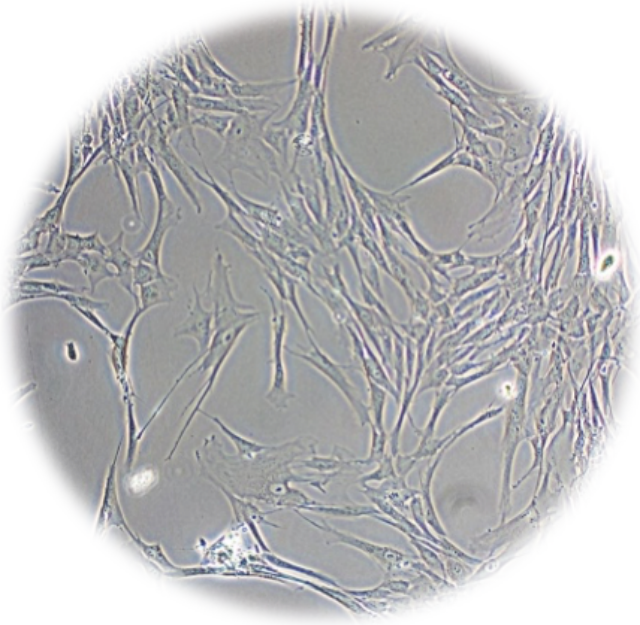

Figure 4. Phase contrast image of human fibroblasts

Achievements and challenges

- All these developments ultimately resulted in a **versatile toolbox** of different **lung cell models** applicable to assess the possible impact of long-term or repeated exposure to ENM.
- The design of **complex (patho)physiological relevant lung cell models** is **challenging** and more work is needed towards the development of reliable and reproducible protocols.
- With an eye on a possible **future guideline**, a co-culture model consisting of bronchial epithelial cells and macrophages (primary/cell lines) is currently subjected to an **interlaboratory comparison** between eight institutes, addressing both ENM deposition and cellular response.

[5] Drasler et al. An Inflamed Human Alveolar Model for Testing the Efficiency of Anti-inflammatory Drugs in vitro. Frontiers in Bioengineering and Biotechnology, 8, 2020.

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