In Vitro Replica of the Inner Surface of the Lungs for the Study of Particle-Cell Interaction

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Background and aim

Adverse health effects by inhaled and deposited particles are of great concern. In addition, therapeutic aerosols become increasingly important for the treatment of lung and other diseases. The interaction of particles with the inner surface of the lungs is fundamental for their biological effectiveness in the organism, though poorly understood and precluded from being studied in humans. A large number of animals are used to test the effects of aerosol particles on a living system by whole-body or nose-only exposures. Such experiments are time consuming

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and they are stressful for the animals. Furthermore, the increase of knowledge in the use of animal models is hampered for various reasons, such as the long time lapse between aerosol application and lung fixation, the lack of methods for direct studies, or artefacts produced by chemical lung fixation. Cell cultures which are in common use today are deficient in important structural components of the inner lung surface, such as macrophages, immune cells, the aqueous lining layer, and the surfactant film at the air-liquid interface. It is the aim of this project to establish a 3-dimensional (3D) primary cell culture system that reflects the differentiated inner surface of human conducting airways to study adverse health effects induced by aerosol particles.

Method and results

The proposed primary cell culture system consists of the main structural components of the inner surface of the conducting airways which are known to acutely react upon inhaled and deposited harmful particles. It contains a differentiated respiratory epithelium with ciliated and secretory cells and macrophages, grown at the air-liquid interface.

1) Epithelial cells are derived from porcine trachea, human bronchi, or from an immortalised human bronchial cell line and expanded in petri dishes. For differentiation, cells are transferred to porous filter inserts in a two chamber system and grown at the air-liquid interface for at least 3 weeks. Further, respiratory epithelia obtained by micro-dissection from porcine trachea are cultured on porous filter inserts and maintained at the air-liquid interface for direct use for aerosol experiments (Fig. 1).

2) Macrophages obtained by broncho-alveolar lavage (BAL) from donor lungs or pig lungs are added on top of the epithelium. Porcine BAL macrophages cultured on filter inserts are shown in Figure 2.

3) Respiratory epithelial cell cultures may not produce their own surfactant at the air-liquid interface. In that case, an artificial surfactant film has to be added.

The integrity of the 3D primary culture system and the state of differentiation are regularly monitored by morphological, physiological, and biochemical analyses.

The 3D primary cell culture system described is used in particle exposure studies to investigate possible health effects by environmental and manufactured nanoparticles. Various particles (different materials, size, and surface) are applied to the cell cultures as aerosols in a specially designed particle deposition chamber. The cellular responses upon the exposure to the aerosols are studied in a time series using light and electron microscopy for morphologic analyses, by ELISA to check for (pro-)inflammatory cytokine release (e.g. IL-6, IL-8 and TNF-α) and for cytotoxicity (e.g. LDH release), and eventually by molecular biological techniques to monitor gene expression patterns. So far, the cell system has been used in exposure studies with polystyrene (test) particles and (secondary) organic aerosols. Data evaluation is in progress.

Conclusions and relevance for 3R

The proposed fully differentiated 3D primary cell culture should reflect the organ-specific functions of the conducting airways. This is an important prerequisite to replace animal models with *in vitro* ones. The suggested model allows studying particle effects at a cellular and molecular level. It may be used in different areas.

1) To assess possible health risks of newly produced nanoparticles and nanotubes.

2) To unravel the health risks by inhaled ultrafine particles generated by combustion processes, e.g. by diesel engines and wood burning stoves.

3) To investigate the health risks of (genetically-modified) microorganisms used in food processing industry.

4) To assess the biological effectiveness of new therapeutic aerosols to treat lung and systemic diseases.

Moreover, in the future, the proposed model system, which will at first represent the situation in the healthy lung, may be replaced by a replica of diseased lungs and, hence, reduce the need for experiments with diseased animal models. Since most

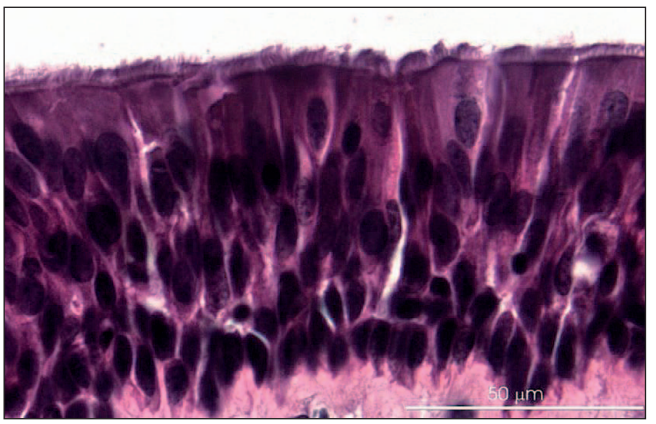

Fig. 1: Light micrograph of differentiated respiratory epithelium obtained by micro-dissection from a porcine trachea cultured for 3 days on filter inserts at the air-liquid interface. Paraffin section, hematoxilin-eosin staining.

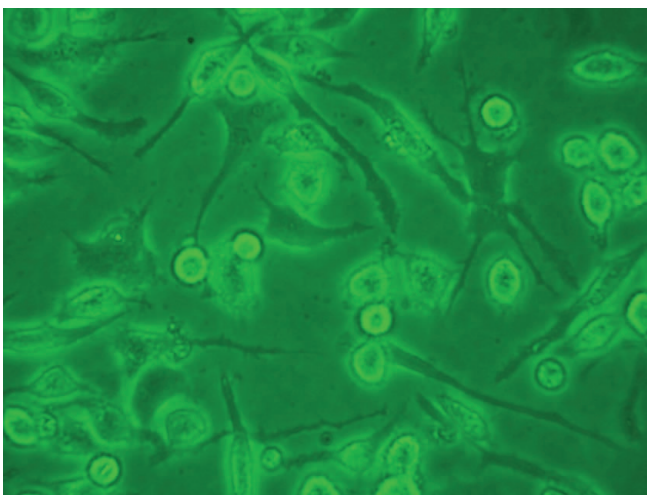

Fig. 2: Porcine macrophages obtained by bronchoalveolar lavage in cell culture. Micrograph of living cells.

animals in inhalation studies are used in the pharmaceutical industry, the introduction of the proposed 3D primary cell culture system in such companies has a large potential for 3R. A successful introduction of the proposed model in industry may reduce a substantial number of painful animal experiments, replace animal experiments by *in vitro* testing and refine *in vitro* model systems used today to study particle-lung interactions.

References

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