Nanostrukturierte Materialien: Gesundheit, Exposition, Materialeigenschaften

Interaction of nanosized SiO_2-FITC-labeled particles with the barriers of the deep lung

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Introduction

Pulmonary surfactant is the first non-cellular barrier an inhaled particle comes in touch with after deposition in the deeper lung. As a consequence, the interaction of the particle with the surfactant components is likely to result in a surface modification which can be important for the further fate of the particle. To evaluate the influence of such “surfactant corona”, experiments were carried out with nanosized FITC-labeled SiO_2-particles, either in plain form or after incubation with pulmonary surfactant, using a new established cell culture model based on primary alveolar macrophages and primary alveolar type I-like pneumocytes. This co-culture model allows addressing epithelial barrier function as well as macrophage clearance in the deep lung simultaneously. Pulmonary native surfactant (nS) was obtained from porcine bronchoalveolar lavage fluid (pBALF) after a sequence of purification steps. The nS was characterized with respect to phospholipid and protein compositions and was in agreement with data reported in the literature.

APQ: Isolating native surfactant from porcine lungs

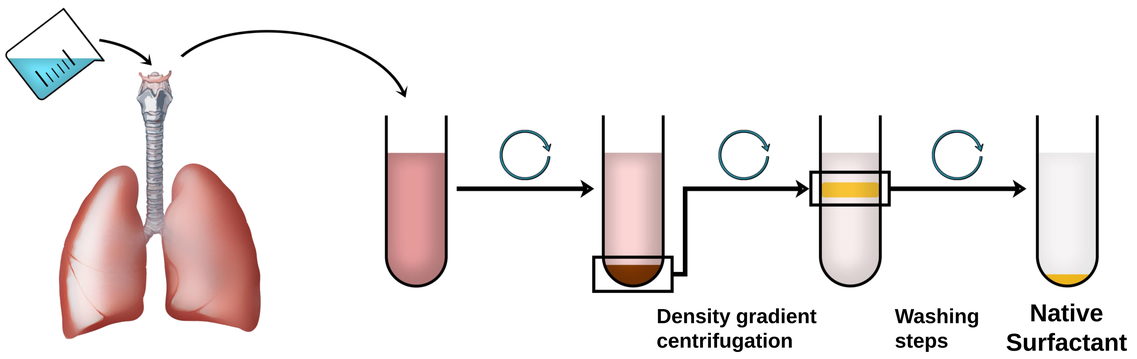
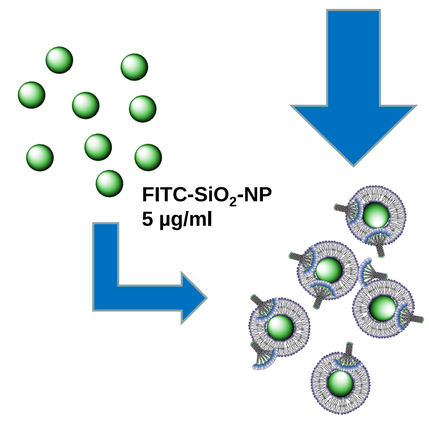


Figure 1: Schematic diagram of the native surfactant (nS) isolation from porcine bronchoalveolar lavage fluid (pBALF)

1.) Bronchoalveolar Lavage was carried out with fresh porcine lungs to obtain porcine bronchoalveolar lavage fluid (pBALF) (Figure 1). By multiple centrifugation steps the native Surfactant (nS) fraction was isolated. It also has been shown that during the isolation steps the total amount of lipids decreases, but the relative composition remains steady and was compared to data found in literature (Table 1).

Table 1: Comparison of the lipid composition of the isolated nS with published values.

Lipid species	Literature [1] w/w	Literature [2] mol% of total PL	Literature [3] mol% of total PL	NanoGEM – nS mol% of total PL
Phosphatidylcholine (PC)	75%	79.5 ±1%	85,6 ±1.8%	85.29%
Phosphatidylglycerol (PG)	10-15%	4%	8.6 ±0.7%	4.66%
Phosphatidylinositol (PI)		15%	2.0 ±0.2%	0.83%
Phosphatidylethanolamine (PE)	n.a.	n.a.	2.9 ±0.8%	8.56%
Cholesterol (Chol)	5-10%	n.a.	n.a.	n.a.
Sphingomyelin	n.a.	n.a.	1.0 ±0.5	0.66%
→ The protocol for isolating natice surfactant shows good comparability to surfactant preparations found in literature				



2.) FITC-labelled SiO_2-nanoparticles were incubated according to DQ 1.2.1 which was established to address the handling difficulties of the lipid rich nS. In brief, 5 µg/ml were homogenized in cell media at 37°C for 2 h at 700 rpm and directly applied to the cell layer after removal of the medium.

AP 3: In vitro study

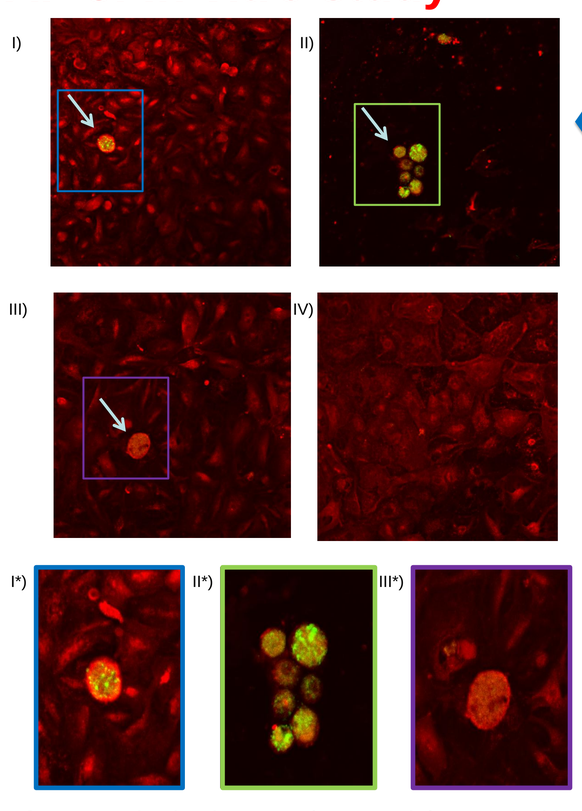


Figure 3. The *in vitro* co-culture model treated with coated (I), uncoated (II) and without particles (III). A monolayer of epithelial alveolar cells incubated with coated particles (green) (IV) does not show any uptake behaviour (III). Cell membranes were stained with RCA I (red).

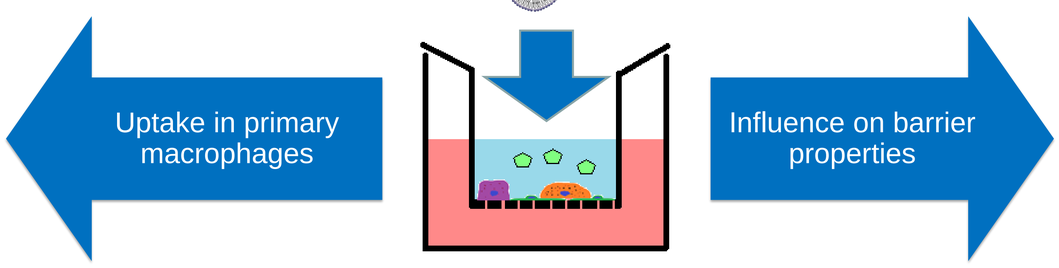


Figure 2: Scheme of the co-culture model on a Transwell© membrane. FITC-SiO_2 particles were applied in the apical compartment.

3.) To mimic the human *in vivo* situation in the air blood barrier as close as possible, a more complex in vitro model was developed in the BMBF project PeTrA. This model is based on a co-culture of human primary alveolar type(AT)I-like cells and human primary alveolar macrophages. The co-culture was treated with coated, as well as non-coated, SiO_2-particles to investigate the uptake behaviour (Figure 3) and barrier functions (Figure 4) *in vitro* and to determine a possible penetration.

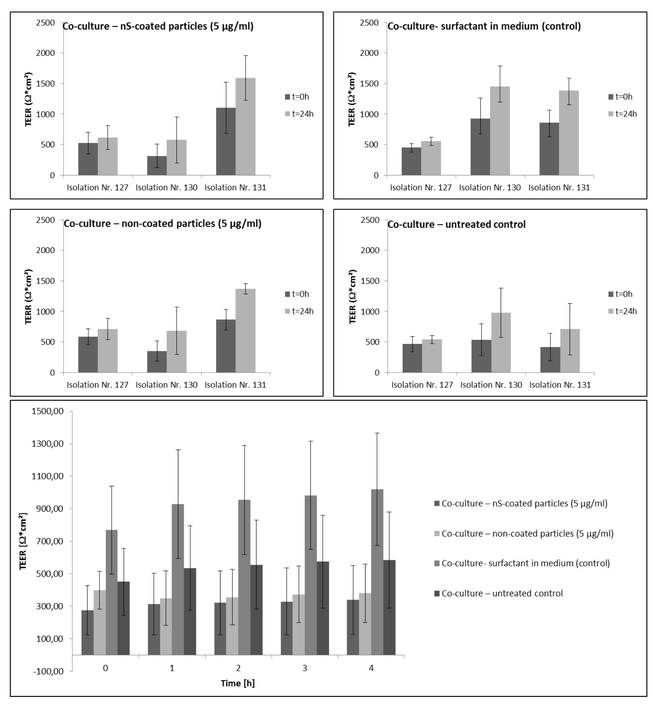


Figure 4. To monitor possible effects on the barrier function, the transepithelial electrical resistance (TEER) was measured repeatedly at different time points. No significant changes were observed under the influence of either type of particle during the experiment. This indicates that there is no penetration of SiO_2-particles by the paracellular pathway (opening of tight junctions). The experiment was repeated with three different isolations (127, 130 and 131).

Results & Conclusion

Native surfactant preparation shows good comparability to surfactant preparations found in literature

Coated, as well as non-coated particles, were internalized by macrophages

No cellular uptake was observed by epithelial cells

No significant changes in TEER-values observed

Effects of the lung surfactant under liquid-interface exposure where less pronounced than expected

References:

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