Epithelial barrier investigations *in vitro* – Application of a primary cell model to study effects of ultrafine particles in human airways

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Introduction

The nasal mucosa represents one of the first interfaces between the environment and the interior of the human organism. It is the first barrier against continuously inhaled environmental pollutants such as particulate matter (PM). The mucociliary clearance represents a very important intrinsic defense system of the nasal epithelial barrier. It can be disrupted by exposure to substances such as PM, which in turn might promote the development of certain diseases like allergies. According to the WHO, PM from road traffic with particle sizes of 2.5 µm and 10 µm ($PM_{2.5}$ and PM_{10}) cause adverse health effects. However, the effects of $PM_{0.1}$ - the ultrafine fraction of PM < 100 nm - are still unknown. The analysis of exposure to PM requires an airway *in vitro* tissue model with high *in vivo*-similarity including mucus production, beating kinocilia, and sufficient barrier properties.

Material and Methods

Our tissue models are generated from human nasal mucosa biopsies obtained during paranasal sinus surgery. These models are cultured under air-liquid interface conditions, mimicking the human upper airway mucosa, including mucus production and beating kinocilia. To further determine the properties and potential of our model, we established transepithelial electrical resistance (TEER) measurements that allow us to analyze the effects of the epithelial barrier upon exposure to ultrafine carbon black particles (UFPc). For this purpose, our mucosal model was exposed to UFPc using a nebulizer ("cloud"). This made it possible to keep the cells at the air-interface during and following exposure. Besides TEER, genotoxic and cytotoxic analyses were performed.

Results

TEER measurements were obtained on day 14 and 21 of co-culture and before and after UFPc exposure. In the differentiated models, preliminary results from three donors indicate normalized TEER values around 750-1000Ω*cm^2 in our setup, although much higher values were also measured. Signs of a UFPc -induced TEER response were detected in one donor from 4 µg UFPc per cm^2. Up to 15 µg of UFPc per cm^2, no effects on cell viability (MTT assay and trypan blue staining) or DNA damage (comet assay) were detected.

Discussion

These data suggest that baseline TEER values in our model are higher than those reported for human respiratory cell lines. Although not completely comparable, monocultures of fibroblasts and epithelial cells in the model setup commonly produce TEER values comparable to the blank control or slightly above, and the additive effect of two cell types thus does not explain the 750-1000Ω*cm^2 in

our co-culture model. We speculate that these higher values are in line with the *in vivo*-necessity for protection against assaults in the respiratory tract, and consider the model valuable to study effects on the respiratory epithelial barrier. If the observed reduction in barrier integrity upon UFPC exposure proves reproducible, it would suggest that a subpopulation of humans are at increased risk of adverse health-effects caused by ultrafine particles.