Development of advanced 3D-models with human primary bronchial epithelial cells (PBEC) for exposure to nanoparticles present in air pollution

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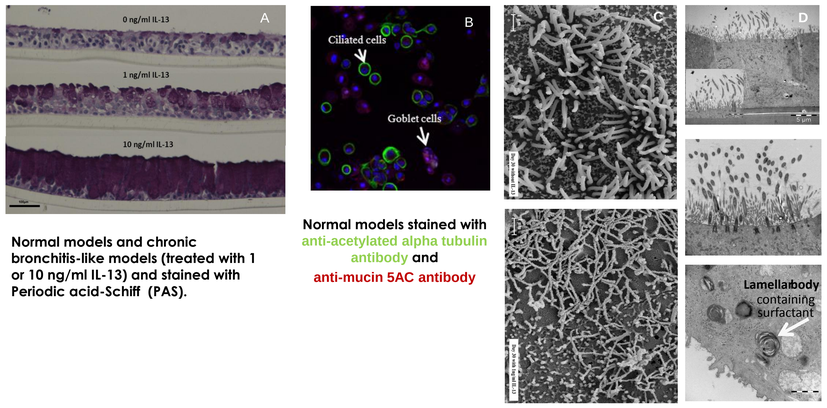
Conclusions

We have developed unique airway wall models, expressing either normal or chronic bronchitis-like morphologies by using primary human bronchial epithelial cells cultured at air-liquid interface.

These sophisticated models with primary human cells combined with realistic exposure with precise dosing mimic the *in vivo* situation and provide useful and relevant tools when studying the effects of nanoparticles present in air pollution.



Normal models and chronic bronchitis-like models (treated with 1 or 10 ng/ml IL-13) and stained with Periodic acid-Schiff (PAS).

Normal models stained with anti-acetylated alpha tubulin antibody and anti-mucin 5AC antibody

Figure 1 Light microscopic (A), confocal microscopic (B), SEM (C) and TEM (D) photos of the models airlifted for 2 weeks

	Normal model	Chronic bronchitis-like model
IL-8 (ng/ml)	22.28 (7.04-38.14)	39.13** (9.42-69.53)
Club cell protein (CC10/CC16) (ng/ml)	3.72 (2.65-15.63)	18.80 (6.51-22.37)
MMP-9 (ng/ml)	15.71 (14.44-18.74)	11.95 (4.98-13.08)

Table 1 Inflammatory mediators from basal medium after 24 hours incubation before nanoparticles exposure.

Normal model and chronic bronchitis-like model treated with 1 ng/ml IL-13.

Results are presented as median and 25th -75th percentiles.

** indicate P<0.01, comparisons between normal and chronic bronchitis-like model (Wilcoxon signed rank t test).

Introduction

Exposures to inhaled nanoparticles, with the lung as the primary target, are of great concern. For both healthy subjects, and subjects with respiratory diseases like chronic bronchitis, our chosen test substrates of highly dispersed nanoparticles present in polluted ambient air are of clinical importance.

Aim

Our aim is to develop unique models combining the use of human PBEC with realistic exposures; a strategy that mimics exposures of the human airway wall. With further development and validation, these models could form part of *in vitro* testing strategy to reduce the requirement for animal inhalation studies.

Material and Methods

Light-, confocal-, scanning (SEM)- and transmission electron microscopy (TEM) and RT-PCR were used to document the appearance of PBEC grown under air-liquid interface culture condition. Both normal- and chronic bronchitis-like models (treated with IL-13) were exposed to clean air or custom synthesized palladium (Pd) nanoparticles at three different concentrations applying the Xpose*ALI* module of the PreciseInhale™ system— 200ng, 400ng and 600ng per insert—then incubated for 8 and 24 hrs (Fig 2). Both apical (AM) and basal medium (BM) were collected. The release of chemo-attractants (CXCL8), Matrix metallopeptidase 9 (MMP-9) and Club cell protein (CC10/CC16) was detect by ELISA. Cell viability and apoptosis was analyzed with trypan blue and the annexin V-PE/7-AAD kit, respectively.

Results

When culturing at ALI the epithelial cells differentiated into ciliated cells, goblet cells, basal cells and club cells identified by mRNA expression, light and confocal microscope, SEM, TEM (Fig 1) and protein secretion (Table 1). The optimal concentration of IL-13 for developing a chronic bronchitis-like model was 1 ng/ml. Furthermore, exposure to Pd nanoparticles induced an increased secretion of IL-8, and chronic bronchitis-like model released significantly more IL-8 than normal model.

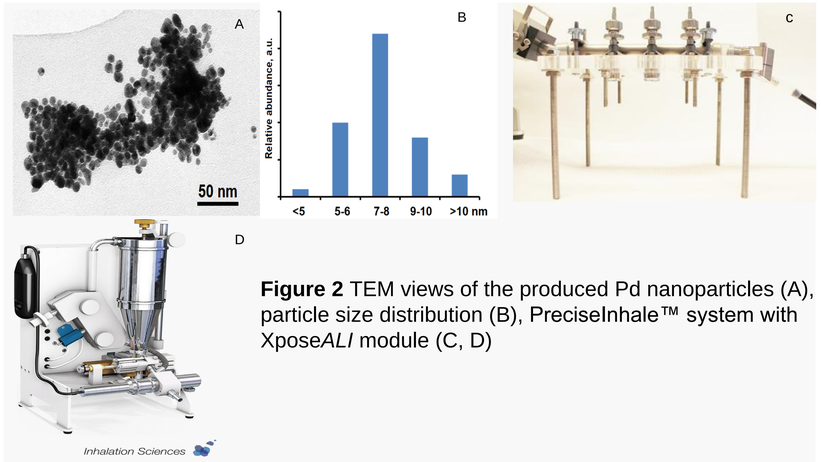


Figure 2 TEM views of the produced Pd nanoparticles (A), particle size distribution (B), PreciseInhale™ system with Xpose*ALI* module (C, D)

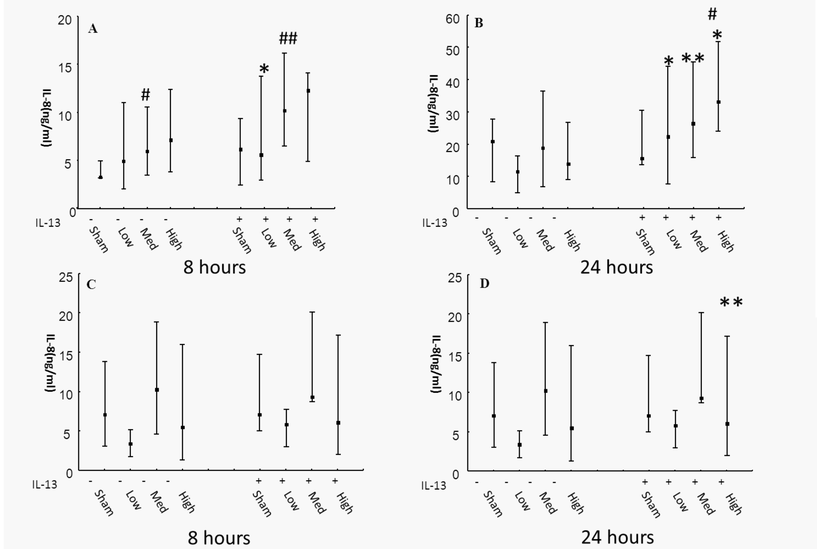


Figure 3 Concentrations of IL-8 levels in basal medium in normal or 1ng/ml IL-13 treated models (N=9) after exposure to different concentrations of Pd nanoparticles and incubated for 8 hours (A) or 24 hours (B). Concentrations of IL-8 levels in apical medium in normal or 1ng/ml IL-13 treated models (N=9) after exposure to different concentrations of Pd nanoparticles and incubated for 8 hours (C) or 24 hours (D). # : P<0.05 VS Sham exposure; *, **: P<0.05, 0.01 VS normal model.

All studies included in this work were approved by the Regional Ethical Review Board in Stockholm.

Per Gerde is a minority shareholder in Inhalation Sciences Sweden AB.

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