Size, number and surface influence the entering of particles in A549 lung epithelial cells

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Introduction

Airborne particles may enter epithelial cells (EC) of the respiratory tract. Little is known about the quantitative relationship between particle size and number or surface taken up by EC. Current literature suggests that entering mechanisms of particles differ between fine particles and nanoparticles (> 0.1μm). More over, the parameters size, number and surface are discussed to be responsible for biological response of particles [1].
Therefore this study investigated the entering of differently sized fluorescent, spherical polystyrene particles (Ø 1μm, 0.5μm, 0.2μm, 0.1μm, 0.05μm) into the human lung EC line A549 in regards to different particle number, surface and concentrations. In relation to biological response and different entering mechanisms of particles, the cells were analysed for changes in total surface area of apical cell membrane.

Conclusion

-> There were significantly less intracellular nanoparticles present in epithelial cells compared to fine particles. This result was found for particle number as well as for particle surface.

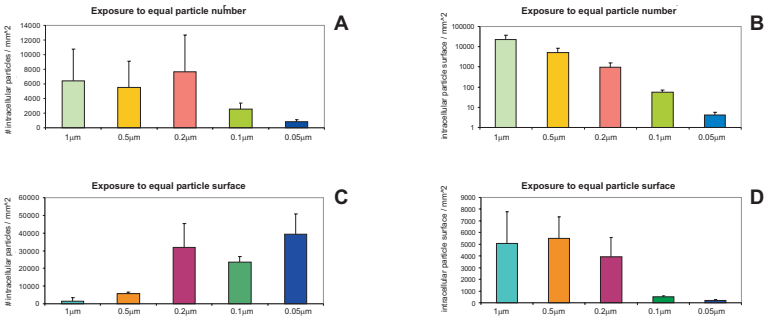
-> Fine particles show a stronger increase of intracellular particle number at rising particle concentrations as compared to nanoparticles.

-> Cells exposed to high concentration of 1μm particle exhibit an increase of total apical cell membrane. The same effect can be observed after exposure to an equivalent particle surface area of 0.05μm particles.

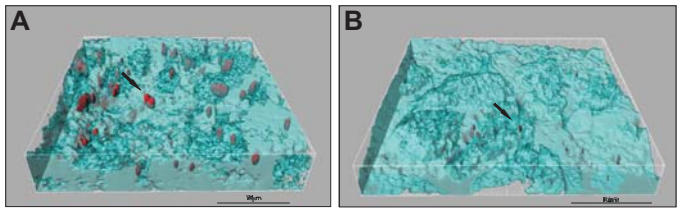
Results

Intracellular particle quantification

Cells were exposed to differently sized particles at either the same particle number (A, B) or the same total particle surface (C, D). Exposure to the same number of particles resulted in significantly fewer intracellular nanoparticles as compared to fine particles ($p<0.05$). This was also observed when EC were exposed to the same total particle surface of differently sized particles ($p<0.05$).



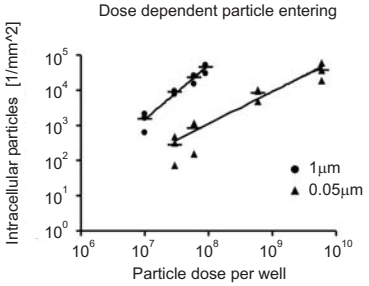
Quantification of intracellular particles was performed by visualization of cells and particles with laser scanning microscopy and numbering particles with a specific counting software.

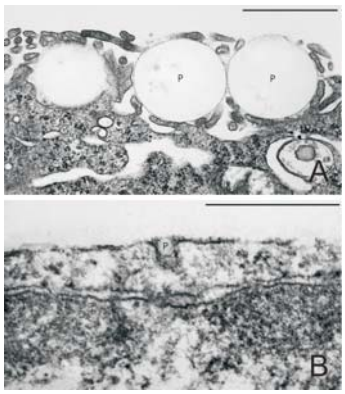


A549 cells with 1μm polystyrene particles (A) and with 0.05μm particles (B).

Concentration and size dependent particle entering

Cells were incubated with different concentrations of 1μm and 0.05μm particles. The number of intracellular particles showed a stronger increase for fine particles as compared to nanoparticles at increasing particle concentrations. Besides, different particle entering mechansims could be observed.

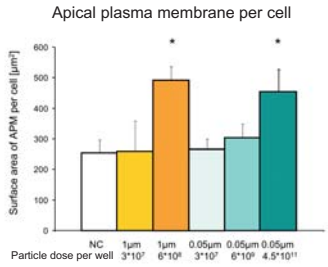




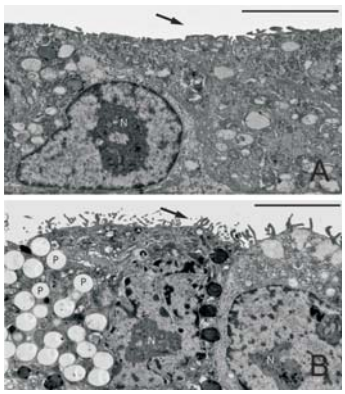
Different entering mechanisms of particles (P). A) 1μm particles entering by macropinocytosis; scalebar= 1μm. B) 0.05μm particle entering by clathrin or caveolae mediated endocytosis; scalebar= 0.5μm.

Apical cell membrane

Evaluation of the total surface area of apical membrane per cell, showed a significant membrane increase ($p>0.05$) after exposure to $6*10^8$ 1μm and $4.5*10^{11}$ 0.05μm particles per cell culture well. These two particle number concentrations have the same total particle surface.



Exposure to low particle dose does not cause any changes in apical plasma membrane compared to the negative control (NC). The exposure concentrations were chosen to correspond in number, surface and mass. The particle dose of $4.5*10^{11}$ 0.05μm has an equal particle surface as the one of $6*10^8$ 1μm particles and an equal mass as the one of $3*10^7$ 1μm particles. The results suggest an effect caused by the total particle surface.



Apical cell membrane (black arrow) of cells exposed to $3*10^7$ 1μm particles per well (A) and to $6*10^8$ (B) where the plasma membrane shows extensions and mickrovilli-like structure. Scalebar = 5μm

Material and Methods

A549 cells were grown confluently and then incubated under submersed conditions for 24h with differently sized spherical fluorescent polystyrene particles (Polyscience, Ø 1μm, 0.5μm, 0.2μm, 0.1μm, 0.05μm). Each experiment was done in triplicate. Particles and cells were visualized with confocal laser scanning microscopy (Zeiss), and after deconvolution with the huygens software (SVI) quantified with a counting software (Dia Count) [2]. For estimation of total apical cell membrane, the incubated cells were further processed for electron microscopy. Stereological evaluation was performed with a cycloid test line system [3].

Literature

[1] Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect*. 2005;113:823-839.
[2] Rothen-Rutishauser B, Muhlfeld C, Blank F et al. Translocation of particles and inflammatory responses after exposure to fine particles and nanoparticles in an epithelial airway model. Part Fibre Toxicol. 2007;4:9.
[3] Muhlfeld C, Rothen-Rutishauser B, Vanhecke D et al. Visualization and quantitative analysis of nanoparticles in the respiratory tract by transmission electron microscopy. *Part Fibre Toxicol*. 2007;4:11.

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