

Laser scanning microscopy combined with digital image restoration to visualize the uptake of ultrafine particles in lung cells

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The uptake of inhaled and deposited ultrafine particles (< 100nm in diameter) in the lung is postulated to cause diseases [1, 2]. In order to study particle-cell interactions, a triple co-culture model of the human airway barrier was designed to simulate the cellular part of the respiratory tract wall represented by macrophages, epithelial cells, and dendritic cells [3]. Since ultrafine particles have the size of small cell components (for example ribosomes), the identification of ultrafine particles in cells is very difficult. Therefore, we combined laser scanning microscopy with digital image restoration to visualize ultrafine particles and to compare their uptake with fine particles (0.1 - 2µm in diameter).

Fluorescent particles, fine (1 and 0.2µm in diameter) and ultrafine (78nm), suspended in culture medium, were added on top of this triple-cell culture which was incubated for 4 and 24h. Thereafter, cells were fixed with paraformaldehyde and stained for F-actin or specific surface markers. The localization of particles was investigated by laser scanning microscopy. For the localisation and visualisation of ultrafine particles at high resolution a deconvolution algorithm was applied to increase axial as well as lateral resolution.

Fine and ultrafine particles have been localised within all three cell types, although dendritic cells were not directly exposed to the particles. Particle uptake was time dependent, since after 24 hours more particles were found in macrophages as well as in epithelial cells than after 4 hours, and also cell type dependent. We never observed as many particles in the epithelial cells as in macrophages and dendritic cells.

Using laser scanning microscopy in combination with digital data restoration fine and ultrafine particles could be visualized in the cells. The three dimensional model is used to study particle uptake and translocation by the three cell types and their possible interplay during this process. More investigations are needed to understand the uptake mechanism and translocation pathways.

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