Translocation of fluorescently labelled SiO_2 nanoparticles across human bronchial epithelial monolayers

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Potential effects of nanomaterials on human health and especially their ability to cross physiological barriers have to be determined because of applications of nanotechnologies in many fields. Inhalation is a frequent mode of exposure to nanoparticles (NPs) and there is evidence of their ability to cross epithelial barriers to reach secondary organs via the bloodstream. Our aim is the study of NP translocation across human bronchial epithelial cells using SiO_2 NPs of three different sizes (16, 50, and 100 nm).

We used the Calu-3 cell line which is able to form an efficient bronchial barrier when seeded onto a porous membrane. By evaluation of the transepithelial electrical resistance (TEER), as well as by monitoring the paracellular transport of a fluorescent marker (Lucifer Yellow), we have shown that Calu-3 cells have the ability to form a tight epithelial monolayer after 2 weeks in culture when they were seeded on 3 µm-polycarbonate Transwell membranes.

Fluorescently labelled SiO_2 NPs (SiO_2-FITC NPs) were used to quantify translocation, and we first investigated their ability to cross the porous membrane in the absence of cells. Whatever the NP size, 24 hours after applying NPs to the apical chamber, the NPs were recovered in the basal chamber with an increasing NP concentration (up to 10%) when the applied apical concentrations were decreasing. By confocal microscopy we observed that the majority of the NPs remained trapped in the membrane. Upon addition of foetal calf serum or dipalmitoyl lecithin, the transport of NPs increased by 5 or 30 times, respectively.

For concentrations up to 10 µg/cm² SiO_2-FITC NPs were non cytotoxic for the Calu-3 cell line and had no pro-inflammatory effect after 24 hours of treatment as shown by studying Interleukine-8 release. However they dose-dependently induced the expression of a specific bronchial mucin, MUC5AC. The translocation of NPs was size-dependent: 6% of the NPs applied to the apical chamber were recovered in the basal chamber when cells were exposed to the 16 nm-sized NPs whereas this percentage fell to 2% for 100 nm SiO_2 NPs. Confocal microscopy experiments were used to confirm NP internalization.

Experiments were also performed on primary Normal Human Bronchial Epithelial cells (NHBE). Quantitative translocation assays cannot be done as these cells must be grown on low porosity membranes (0.4 µm pores). Only morphological studies by confocal microscopy were performed on NHBE cells which exhibit normal (mucociliary) or pathological (squamous and mucosal) differentiation. Preliminary results have shown that NP retention inside the monolayer was differentiation-dependent with a very important retention inside the mucosal epithelium.

In conclusion, SiO_2-FITC NPs can cross in a low amount the bronchial epithelial barrier at non cytotoxic concentrations, without inducing cellular inflammation and affecting epithelial integrity. The mechanisms involved in this translocation are now under investigation. The Calu-3 cell line revealed to be a suitable model to characterize the ability of NPs to cross epithelial barriers according to their physico-chemical characteristics.

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