Preferential intracellular localization of nanoparticles, evaluated by stereology

C.Brandenberger[1], B.Rothen-Rutishauser[1], A.-G.Lenz[2], O.Schmid[2], P.Gehr[1] and C.Mühlfeld[3]

[1]Institute of Anatomy, Histology, University of Bern, Switzerland
[2]Institute of Inhalation Biology, Helmholtz Zentrum München, Germany
[3] Institute of Anatomy and Cell Biology, Justus-Liebig-University Giessen, Germany

brandenberger@ana.unibe.ch

Keywords: Stereology, Nanoparticles, Intracellular Particle Trafficking, Relative Deposition Index, Electron Microscopy

Nanoparticles (NPs) may enter cells by various mechanisms and subsequently be distributed in different cellular compartments. Despite numerous studies on the uptake mechanisms of NPs, no quantitative data is available relating the number of NPs to the relative size of the associated cellular compartments. In this study the preferential intracellular localization of gold nanoparticles has been quantitatively evaluated by the stereological method of the relative deposition index [1]. We analyzed whether exposure duration, agglomerate size or partial inhibition of endocytosis have an effect on intracellular NP distribution.

A549 cells were exposed at the air-liquid interface to 15nm gold NPs. Partial inhibition of endocytosis was achieved by methyl-β-cyclodextrin. At 1h, 4h and 24h after exposure, cells were processed for transmission electron microscopic analysis. Relative intracellular NP distribution was estimated by stereology (Figure 1) and tested with the χ^2-test. Experiments were repeated three times and a total of 2090 NP events was counted.

A significant non-random distribution was revealed at all time points. Most of the counted NPs were localized in vesicles (Figure 2) of different size categories (>150, 150-1000, >1000nm), only 24 NPs were found free in the cytosol, but non in golgi apparatus, endoplasmatic reticulum, mitochondria or nucleus. Comparing the different exposure times, a significant shift of NP localization from small (<150nm) towards large (>1000nm) vesicles occurred between 4h and 24h. Agglomerates >100nm were predominantly localized in middle and large sized vesicles. The blockade of caveolin- and clathrin-mediated endocytosis resulted in less observed intracellular NPs and a significant preferential localisation in large vesicles.

The non-random intracellular distribution of gold NPs and the main localisation in membrane bound vesicles suggests that gold NPs are predominantly taken up by endocytotic pathways. The inhibition study indicates that different endocytotic mechanisms are involved.

[1] C. Mühlfeld et al., J. Aerosol Med. **20** (2007) p395
[2] This research was supported by the Deutsche Forschungs Gemeinsschaft (DFG), the Doerenkamp-Zbinden Foundation, and AnimalFreeResearch

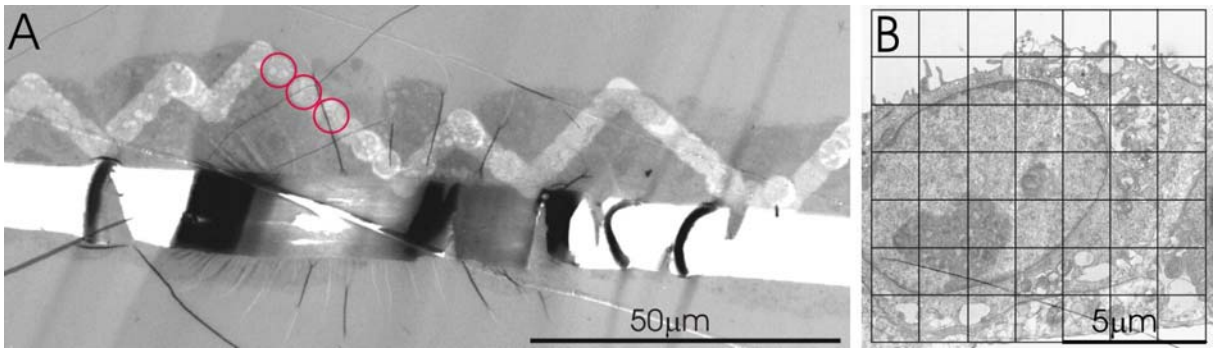

Figure 1. Stereological evaluation of the intracellular particle localization: Ultrathin sections were screened for particles by systematic random sampling (A) and each particle event was reported. The relative size of each cell compartment was estimated by counting the number grid point hitting each cellular compartment (B).

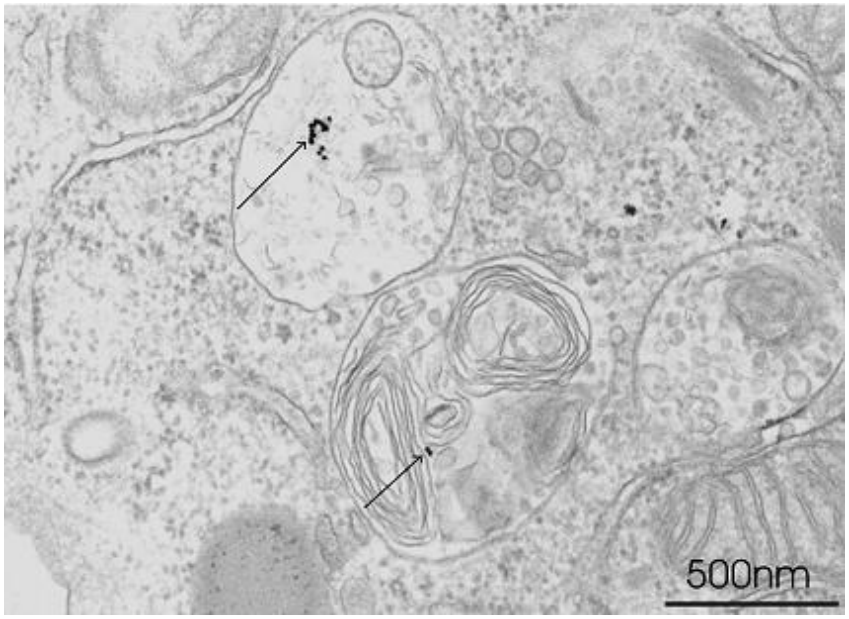

Figure 2. Intracellular particle localization: A significant non-random distribution of intracellular particles was revealed. The particles (arrows) were preferentially localized in intracellular vesicles of different sizes.

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-318, © Verlag der TU Graz 2009