Influence of size and surface properties of particles on translocation into cells and on cellular behaviour

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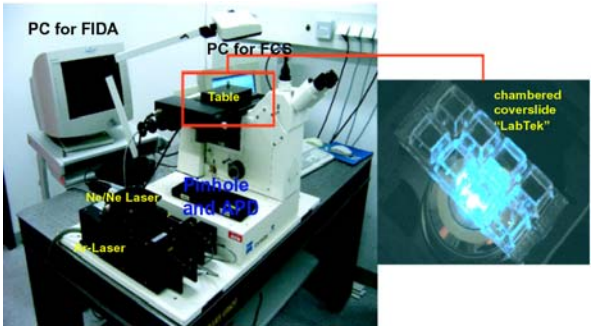
Abstract

LSM images and measurements with Fluorescence Correlation Spectroscopy (FCS) or Photon Counting Histogramming (PCH) showed that negatively charged polystyrene particles with a diameter of 20 nm are effectively internalized by human HeLa cells independent of whether the cells were treated with cytotoxins, interfering with cellular processes, or not. Translocation of particles needs a viable, fluid membrane, as particles are not found within dead cells. Therefore next steps included an extended evaluation of the dependencies of translocation mechanisms on surface properties or size of particles or on used cell lines. An additional issue was the evaluation of cell physiological consequences of the exposure to nanoparticles (Proliferation, metabolic activity, ...).



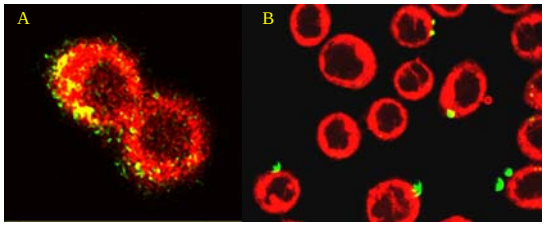
Materials and Methods

- LSM:

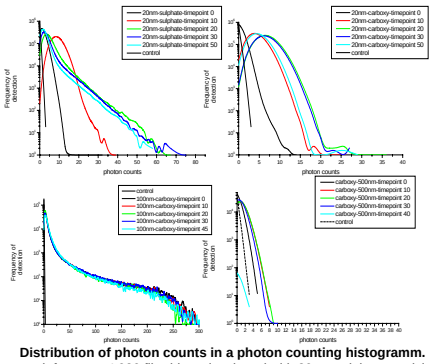
Live cell confocal images were performed using a confocal laser scanning microscope (Leica SP1, Germany), a CW Ar^+ laser and an oil immersion objective (PL APO 100x1.4 OIL UV). Images for the fluorescent particles were performed using the laser line at λ = 488nm. Images for the stained cells were performed using the laser line at λ =568nm. Imaging started 20 minutes after adding the particles and all images were averages of 3 individual scans. Images were extracted and overlays were performed using the Leica Simulator Software (Leica, Germany) or Adobe Photoshop 6.0.

- **FFS (FCS and PCH)**

To obtain raw data for PCH, fluorescence signals were detected using an APD coupled with a Time Measurement Histogram Accumulating Real-Time Processor (PC Board for Time Correlated Single Photon Counting, Time Harp 200, PicoQuant, Software version 3.0) triggered with 7.4MHz. The histogram of counted photons is acquired by monitoring the signal intensity during 30 seconds and plotting the numbers of photons detected in a time window of 40µs.

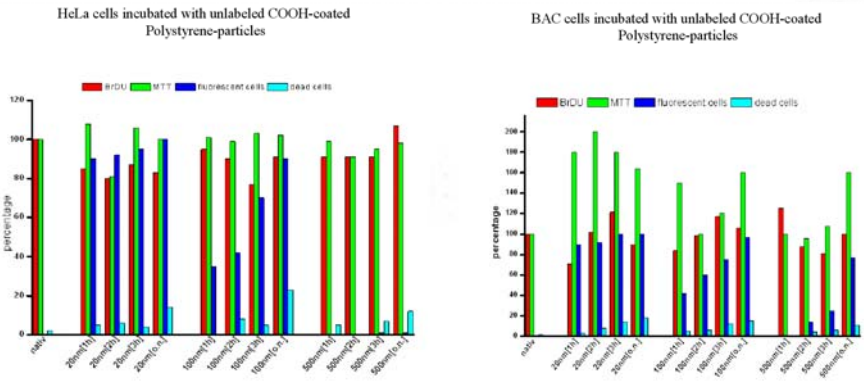


LSM images of BAC cells incubated with negatively charged green fluorescent polystyrene particles.
(A) Cells incubated with 100nm particles.
(B) Cells incubated with 500nm particles.
Images represent sections of 500nm thickness taken 3-4µm above the glass surface



Distribution of photon counts in a photon counting histogramm.
top-left: L929 fibroblasts incubated with 20nm sulphate particles.
top-right: L929 fibroblasts incubated with 20nm carboxy particles.
bottom-left: L929 fibroblasts incubated with 100nm carboxy particles.
bottom-right: L929 fibroblasts incubated with 500nm carboxy particles.

Cells incubated with 20 and 100nm particles show very bright signals in contrast to the control. Cells incubated with 500nm particles do not show such an increase in brightness



Viability assays using epifluorescent analysis (rate of uptake/ dead cells using probidium iodide) and biochemical assays like BrDu and MTT (proliferation and metabolic activity) .
Left: HeLa cells incubated with white carboxy coated particles
Right: BAC cells incubated with white carboxy coated particles
Incubation for 1, 2 and 3 hours and overnight (o.n. 16 hours)
SD = 8-9%
Reference: untreated cells incubated with BrDU and MTT to determine proliferation and metabolic status (set as 100%).

Results

It could be shown that translocation processes are reduced using negatively charged particles with larger diameters (100, 200 and 500nm). If particles reach 500nm in diameter these processes are almost blocked. No significant influence of surface properties on the translocation was found. Sulphate Fluospheres which are relative hydrophobic, due to their reduced charging densities, and amine coated Quantum Dots (data not shown) showed translocation behavior, measured with Fluorescence Correlation Spectroscopy and Photon Counting Histogramming, comparable to the standard used, carboxylate modified Fluospheres.
As there was no significant change in translocation processes the influence of negatively charged particles with selected diameters (20, 100 and 500nm) on cellular processes (cell proliferation, metabolic activity, viability) was evaluated. Additionally these experiments were performed using different cell lines (HeLa – human, BAC - murine). Whereas HeLa cells did not show a significant change in cellular behaviour BAC cells were influenced. On the one hand side they showed a strong increase in metabolic activity if they were incubated with 20nm particles but this effect was reduced using larger particles. On the other side their proliferation was reduced using 20nm particles but almost unchanged using 500nm particles.