**Time-dependent appearance of nanometer-sized particles in living cells**

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### Abstract

Possible negative health effects became an issue in environmental sciences as nanoparticle matters appears also in ambient air as a result of a variety of technological processes. Especially the PM2.5 fraction of these emissions, which consist mainly of carbon associated with metals, oxygen, and organic matter, is assumed to threaten public health and even heritable effects are possible. Nanoparticles and ultrafine particles are also an issue in job safety and therefore global research activities are focused on different kinds of aerosols sources, such as coal fly ash and diesel exhaust particles from modern combustion and fabrication processes. Exposure workers (e.g., road or tunnel construction-site) carry an incalculable risk to contract physiological sequels due to the exposure. A detailed understanding of their interaction with cells is a prerequisite for the appraisal of hazardous effects. The general and unspecific uptake was shown with conventionally used fluorescence detection methods like Laser Scanning Microscopy (LSM). Fluorescence correlation methods were applied to follow the time course of the translocation of the particles within the cell. The experimental results demonstrated that singular particles enter the cell without significant contribution by endocytotic mechanisms and are distributed within the cytoplasm. Subsequently aggregation is observed, which can be blocked by cytoxins, like Genistein and Cycloheximide B, interfering with cellular uptake processes. The observed non-active uptake is due to non-specific interactions with the cell surface and could be responsible for distribution of nanometer-sized materials in tissue.

### 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 10×/4 OIL UV). Images for the fluorescent particles were performed using the laser line at ~488nm. Images for the stained cells were performed using the laser line at ~633nm. 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 6.5.

- **FCS (FCS and PCH)**: For FCS the APD is coupled to a fast digital ALV8800E correlator card that calculates the real-time autocorrelation function. Fluorescence signals were detected for 42 seconds with 3 seconds interval and 12 seconds correlator scaling. The auto correlation was fitted using FCS Access version 1.6.12 (Evotec, Germany) applying a structural parameter of 5.6.

- **FFS (FFS)**: To obtain raw data for FCS, fluorescence signals were detected using an APD coupled with a Time Measurement Histogram Accumulating Real-Time Processor (PC Board for Correlated Single Photon Counting, Time Hary 200, PicoQuent, 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.

### Results - Conclusions

- **LSM**: LSM images showed that negatively charged polystyrene particles with a diameter of 20 nm are effectively internalized by human HeLa cells independent whether the cells were treated with Genistein or not. In native cells isolated particles and small aggregates were found throughout the cytoplasm. Additionally bright aggregates were found mainly at the periphery of the cell. Such aggregates of individual particles could result from incorporation into endosomes or similar structures (e.g. lysosomes, exosomes, multivesicular bodies). In cells treated with Genistein only small aggregates were found near to the cytoplasmic membrane. One explanation could be that in that case particles are not efficiently packed into vesicles or that such structures are not actively transported within the cell. Translocation of particles results a viable, fluid membrane as particles are not found within dead cells.

- **FCS**: FCS identified highly mobile particles with a hydrodynamic radius of about 100 nm. The photon distribution of fluorescence 20nm polystyrene nanoparticles in HeLa cells were performed using the laser line at ~488nm. 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 6.5.