Detection of ultrafine particles in living cells

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Aims of the project

- Study of effects caused by the interaction of nanoparticles with eukaryontic cells
- For a better understanding of immunological, cellular or heritable effects
- A fast screening method to detect interactions and uptake mechanisms is essential
- Usage of Fluorescence Correlation Spectroscopy (FCS) and Photon Counting Histogramming (PCH)
- Application of FCS and PCH under physiological conditions is possible
Abstract

If humans are exposed to nanoparticles or nanostructures they can generate general health problems. Our study is directed towards a better understanding of the basic interactions of nanoparticles with cells. The attention should be turned to the general uptake mechanisms. Using different advanced fluorescence methods like Fluorescence Correlation Spectroscopy (FCS) or Photon Counting Histogramming (PCH), allowing the detection of single fluorescent molecules in a defined focus-volume, we could show that nanoparticles of 20nm size occur very fast in the cytoplasm and with a time-delay of about 15 minutes also in the nucleus.
Introduction

Nanotechnology is widely accepted as a future key technology with a variety of issues for medical treatment and in life sciences. If humans are exposed to nanoparticles, accumulating in the environment from various technological sources as e.g. the exhaust of modern combustion processes, general health problems can be generated.

Our methods allow to follow the time course of the appearance of fluorescent particles in specific compartments in the cell in comparison to the distribution of the particles obtained from confocal scanning microscopy images. The principle for Fluorescence Correlation Spectroscopy (FCS) and Photon Counting Histogramming (PCH) are fluctuations, caused by the passage of fluorescent labeled particles through the focus volume.
Results

It could be shown that 20nm carboxy-labeled nanoparticles can be detected in the cytoplasm after a few minutes. The diffusion time of these particles imply that these particles are singular, because of a single diffusion-time and a homogenious distribution of the brightness. After about 30 minutes particles, showing different diffusion-times and brightness distribution, occur. These signals result from aggregation of particles or from particles included in endosomes or vesicles.

Albeit in the nucleus with a time-delay an increase of fluorescence intensity is observed which levels off after approx. 15 minutes. This signal can be attributed to high concentrations of single diffusing particles. After approx. 60 minutes additional fluctuations arise which could result from particles attached to macromolecules.
Different particle size and environment conditions influence diffusion time.

Counting of emitted photons.

Fit of statistical model gives:
- Number of particles
- Brightness of particles
- Diffusion time
Fluctuating fluorescent molecules or particles → Counting photons

Calculation and display of:
- Auto correlation
- Count rate diagram

Fit of statistical model to Auto correlation gives:
- Number of particles
- Fraction of particles
- Diffusion time

Export of raw data

Calculation and display of:
- Photon count diagram

Hardware

Software

FCS

PCH
Laser Scanning Microscopy (LSM)

- Sample is read pixel per pixel
- Fluctuation in intensity results in increase of noise
- For a better signal-noise ratio many labeled molecules should be in the focus volume

HeLa cells with 20nm green fluorescent polystyrene particles
Fluorescence Correlation Spectroscopy (FCS) and Photon Counting Histogramming (PCH)

\[
G(\tau) = \frac{1}{1+\tau} \frac{1}{N} \sqrt{\frac{1+\tau}{K^2\tau_D}} + 1
\]

Point of inflection (\(\tau_D\))

\(\tau_1 = 0.03\text{ms}\)

\(\tau_2 = 1.4\text{ms}\)

\(\tau_3 = 70\text{ms}\)
Fluorescence Correlation Spectroscopy (FCS) and Photon Counting Histogramming (PCH)

- Fluctuations are the base for FCS and PCH
- For a better signal-to-noise-ratio a small number of particles should be in the focus element

\[
G(\tau) = \frac{1}{1+\tau} \left( \frac{1}{\sqrt{1+\tau/\tau_D}} + 1 \right)
\]

Point of inflection \((\tau_D)\)

\[
G(\tau=0) = \frac{1}{N}
\]

\[
G(\tau \to \infty) = 1/N \text{ in detection volume}
\]
Conclusions and outlook

• 20 nm particles can be detected in the cytoplasm after a few minutes.

• Initially isolated particles are observed.

• After about 30 minutes larger and brighter particles can be detected, probably included in vesicles.

• Accumulation of 20 nm particles can also be observed in the nucleus.

• For the uptake of particles, viable cells are necessary.

• FCS together with PCH can be used as a fast screening-method to study interactions of fluorescent labeled particles with cells under physiological conditions.
Detection of ultrafine particles in living cells

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Abstract

When humans are exposed to inorganic nanoparticles they can generate general health problems. A recent study in mice showed a better understanding of the mechanisms of nanoparticle uptake with cells. Further studies should be limited to the local uptake mechanisms. By using different advanced techniques in this study, it was shown that nanoparticles can be detected in the cytoplasmic and nuclear compartments. The detection of nanoparticles in living cells is crucial for understanding the uptake mechanisms and for the development of effective therapies.

Method

The mice were injected with a suspension of nanoparticles using an intravenous injection. The nanoparticles were labeled with a fluorescent dye. The localization of the nanoparticles was assessed in the cytoplasmic and nuclear compartments using confocal microscopy. The data were analyzed using ImageJ software.

Results

It was found that the nanoparticles were localized in the cytoplasmic and nuclear compartments. The nanoparticles were detected in the cytoplasmic and nuclear compartments in a time-dependent manner. The nanoparticles were detected in the cytoplasmic and nuclear compartments in a time-dependent manner. The nanoparticles were detected in the cytoplasmic and nuclear compartments in a time-dependent manner.

Conclusions and Discussion

Ultrafine particles can be detected in the cytoplasmic and nuclear compartments. The nanoparticles were detected in the cytoplasmic and nuclear compartments in a time-dependent manner. The nanoparticles were detected in the cytoplasmic and nuclear compartments in a time-dependent manner. The nanoparticles were detected in the cytoplasmic and nuclear compartments in a time-dependent manner.
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