Cellular uptake and toxicity of positively and negatively charged silica nanoparticles in A549 human lung cells

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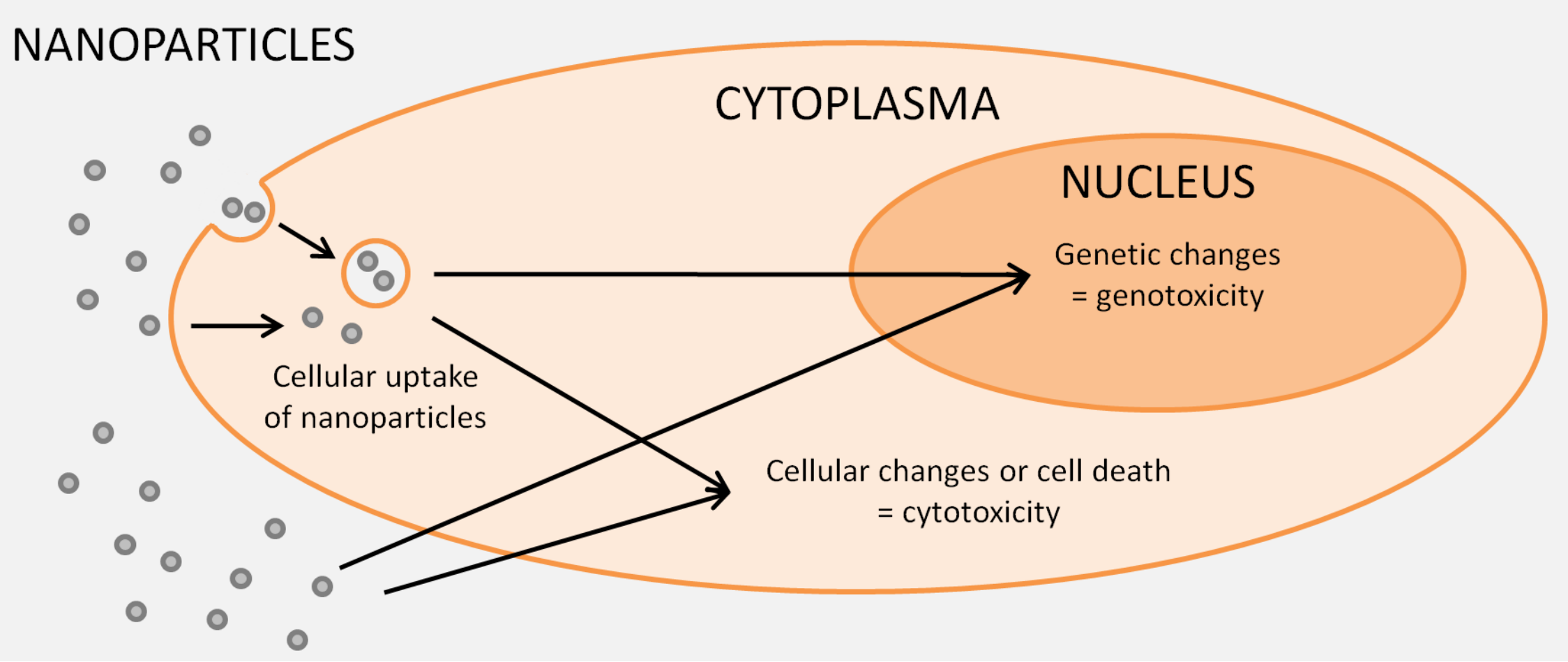
Silica nanoparticles and lung cells



Aims of the study

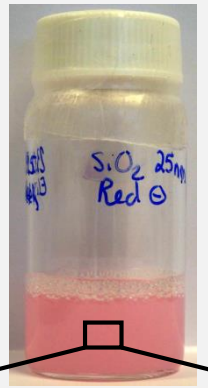
- To identify the effect of silica nanoparticle surface charge on the cytotoxic and genotoxic effects in A549 cells.

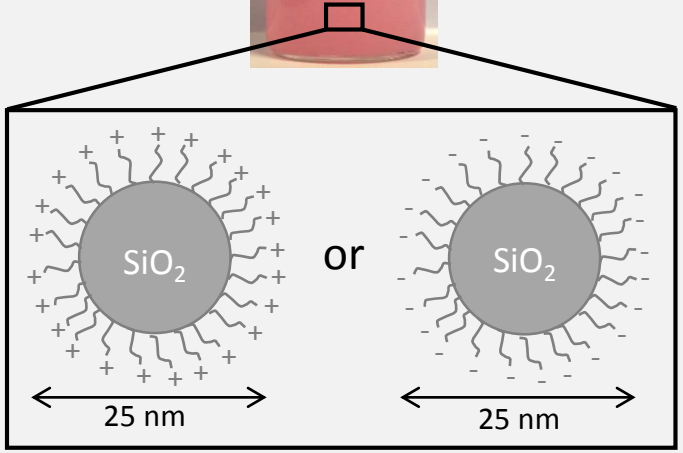
- To identify the effect of silica nanoparticle surface charge on the cellular uptake in A549 cells.

Materials and methods

Characterization:
- **Transmission electron microscopy**
- X-ray energy dispersive spectroscopy
- **Dynamic light scattering**
- Laser Doppler velocimetry
- Nanoparticle tracking analysis

NANOPARTICLES
1 – 300 µg/ml





Raw material provided by Instituto Italiano di Tecnologia (Lecce, Italy)

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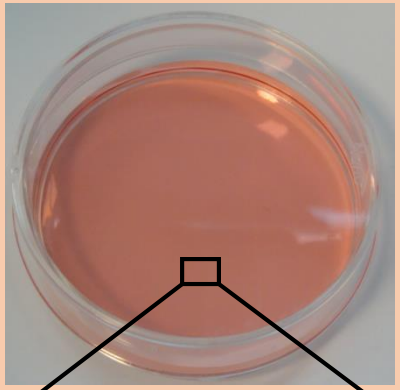
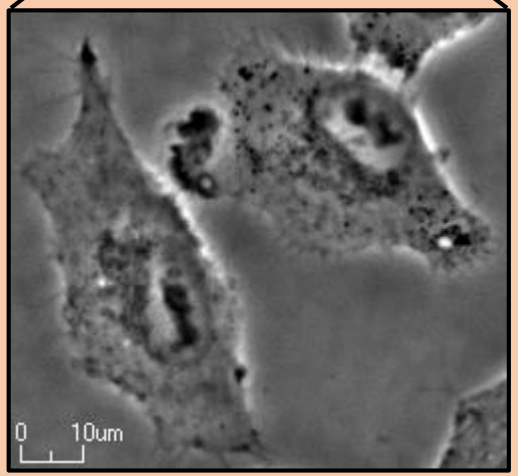
A549 HUMAN LUNG CARCINOMA CELLS





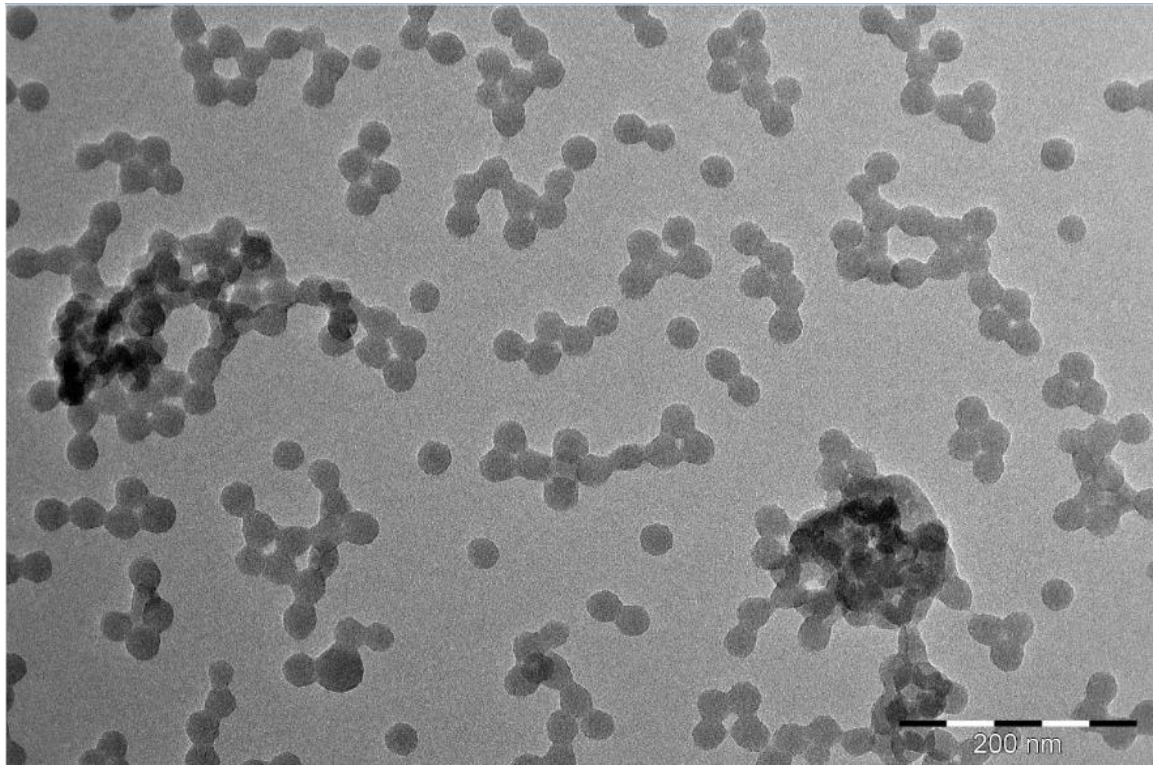
Cytotoxicity:
- **Impedance-based cell monitoring**
- Colony forming efficiency

Genotoxicity:
- Comet assay
- Mouse lymphoma assay (L5178Y TK +/- cells)

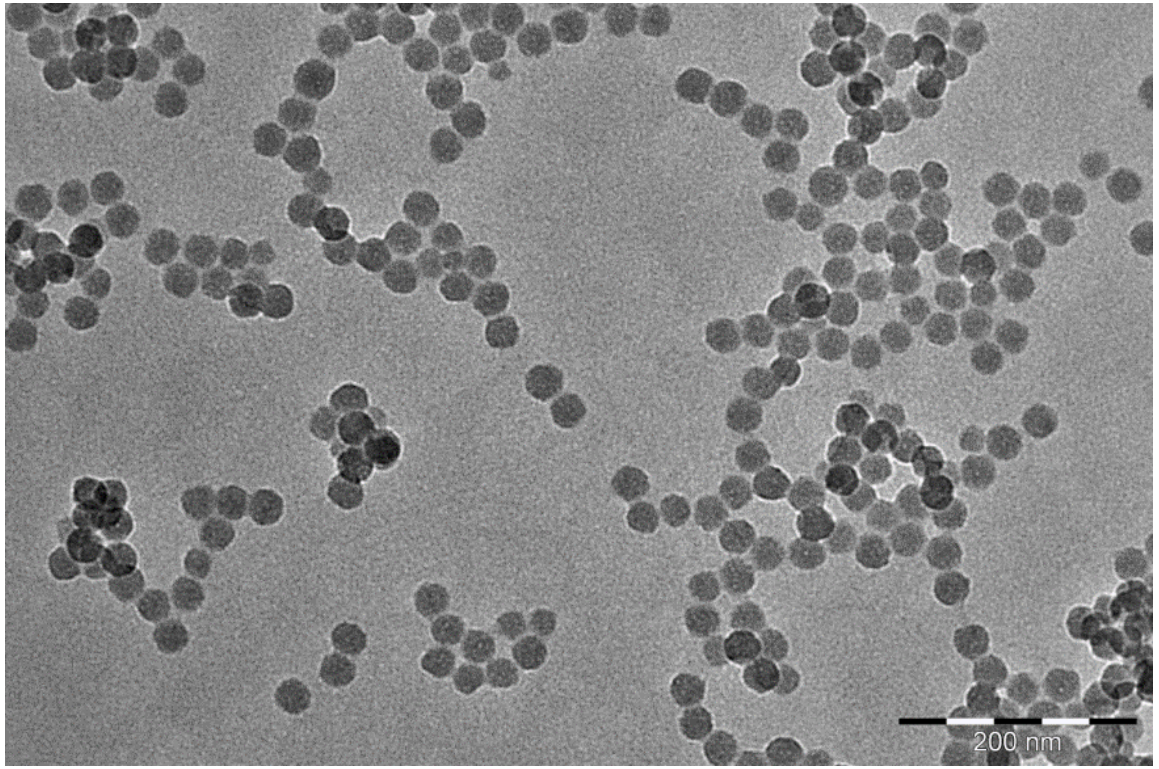
Cellular uptake:
- **Live cell imaging**
- **Flow cytometry**

The NPs show similar characteristics

Positively charged silica NPs



Negatively charged silica NPs



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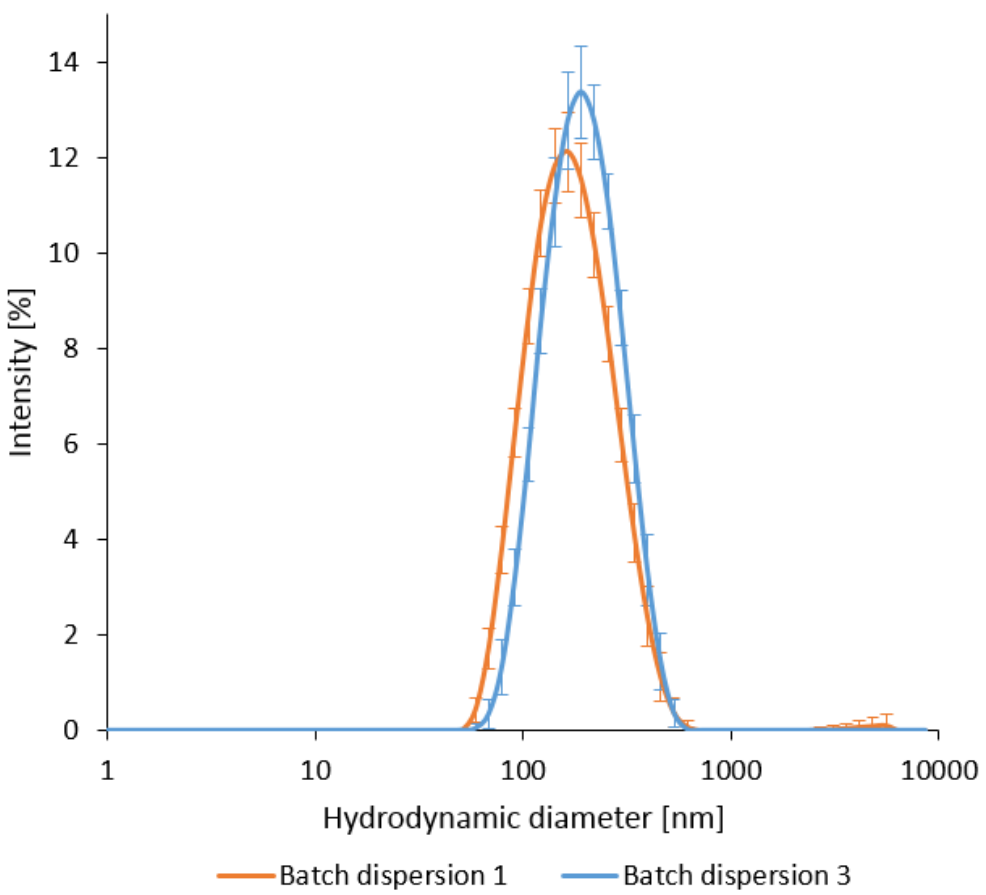

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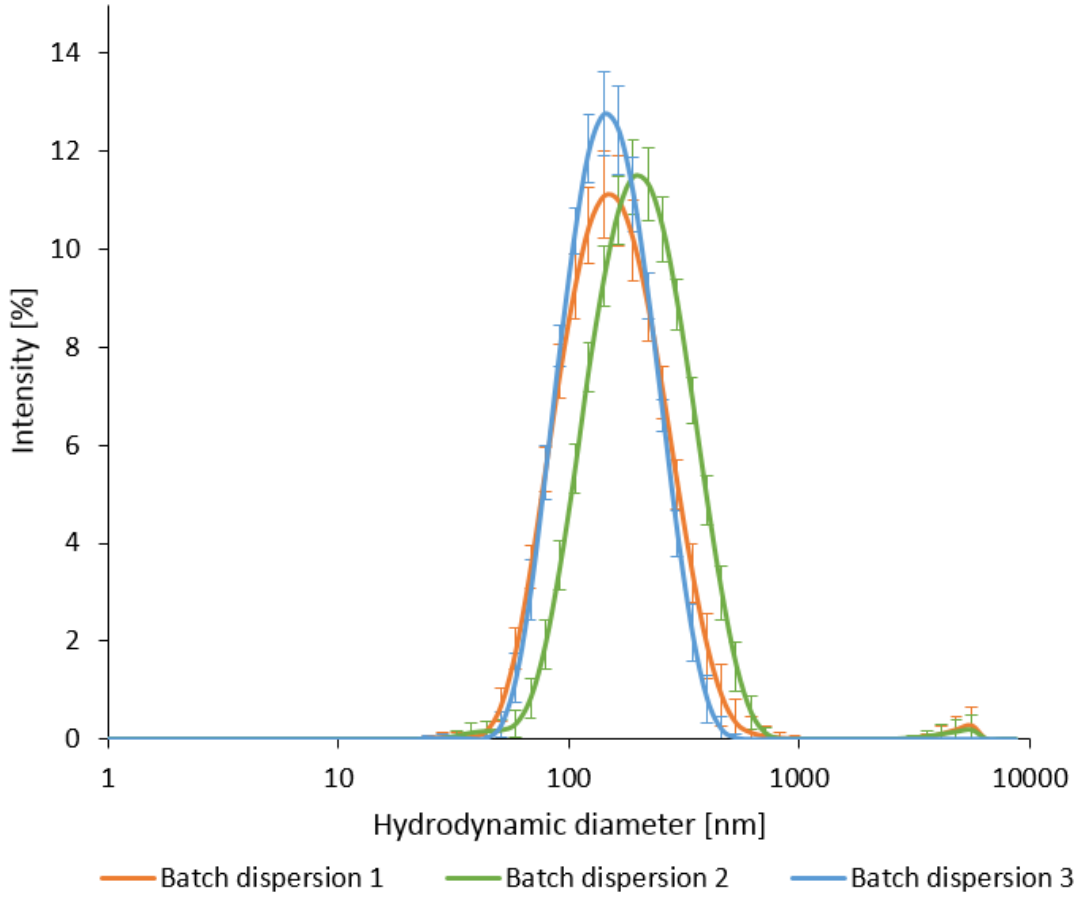
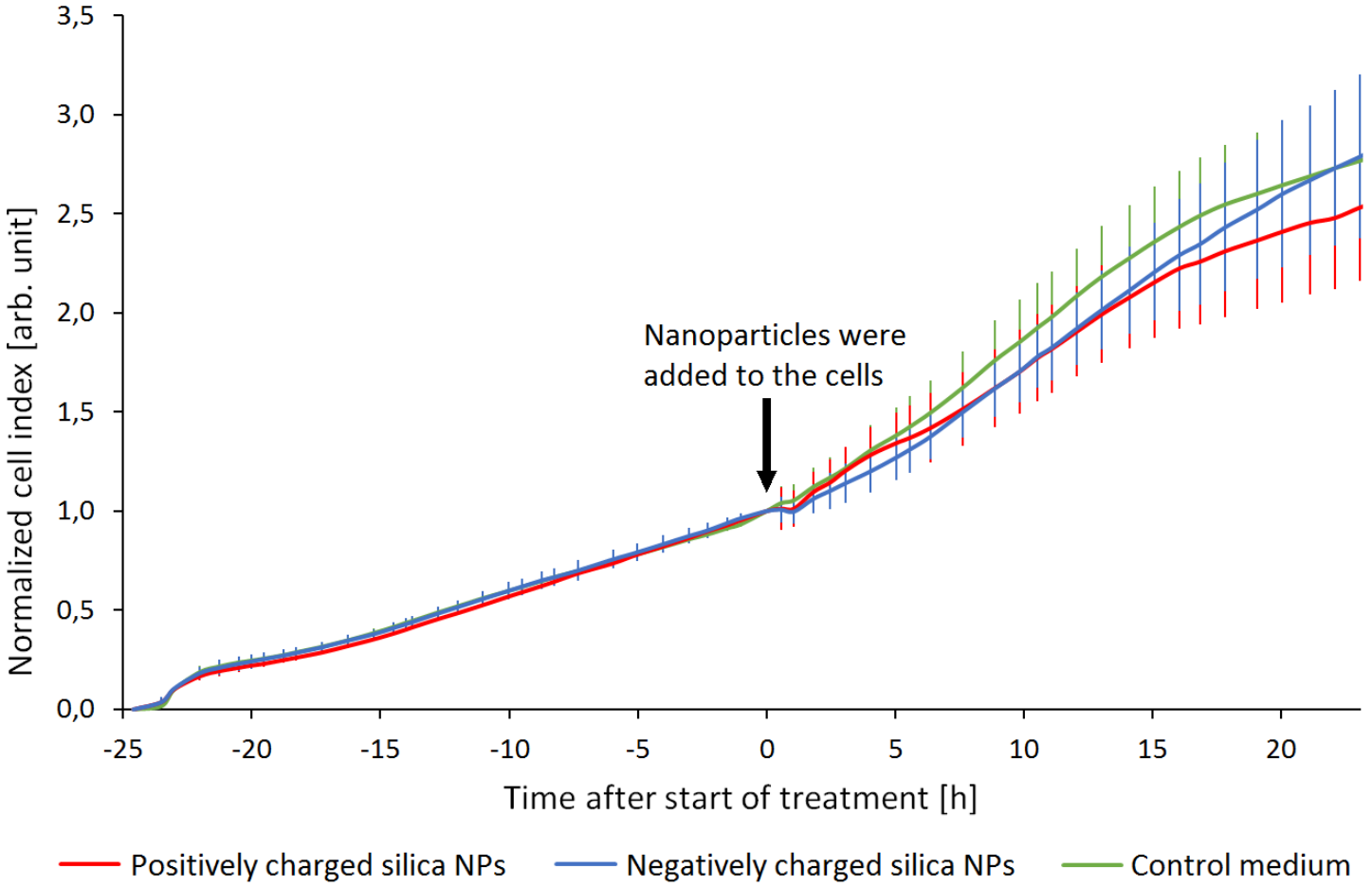


No detected toxicity

Cytotoxicity:

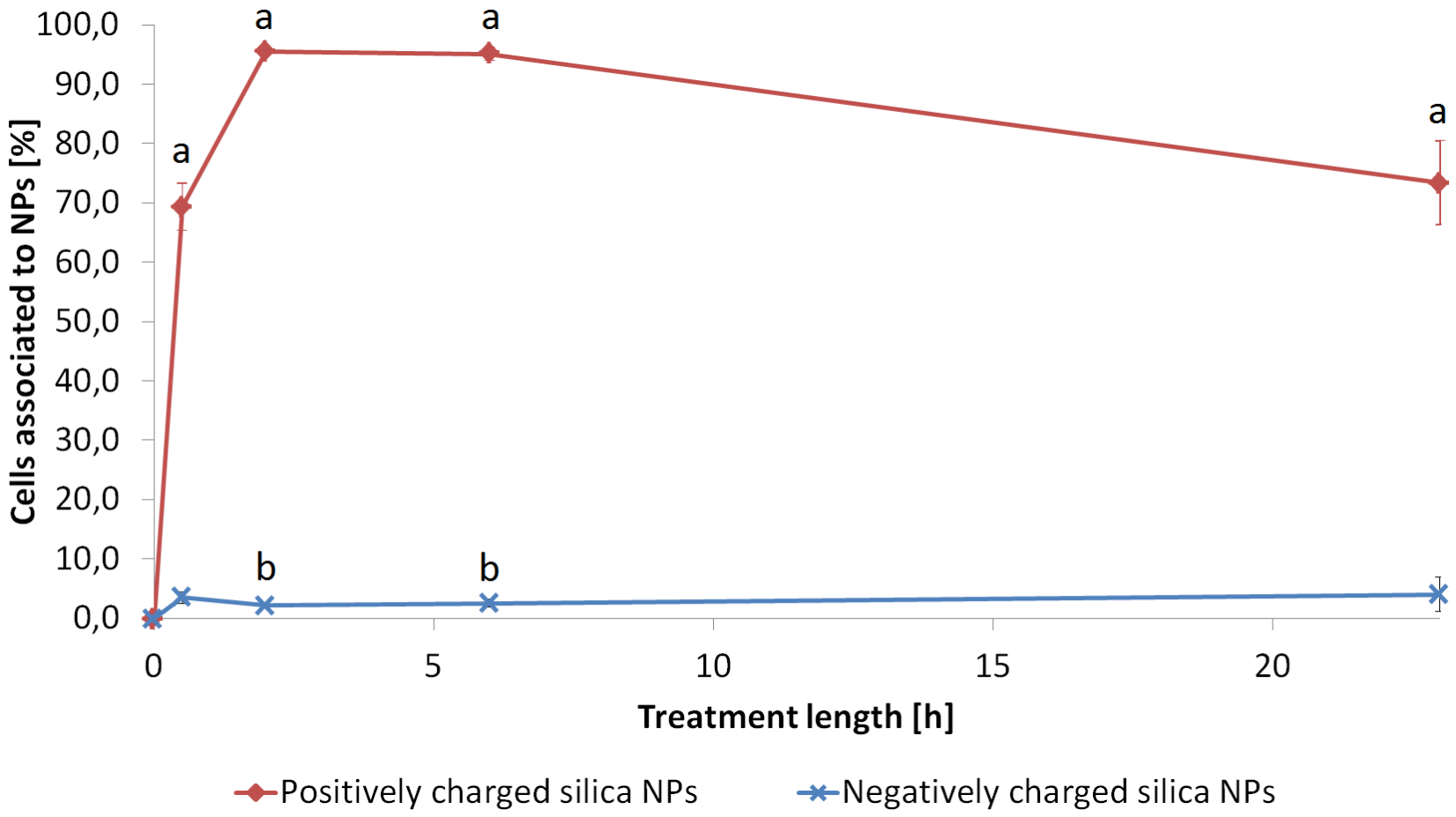
- No cytotoxic effect (by impedance-based cell monitoring, 20 µg/ml)



Genotoxicity/mutagenicity:

- no DNA damage (by the comet assay)
- no DNA oxidation (by the comet assay)
- not mutagenic (by mouse lymphoma test)

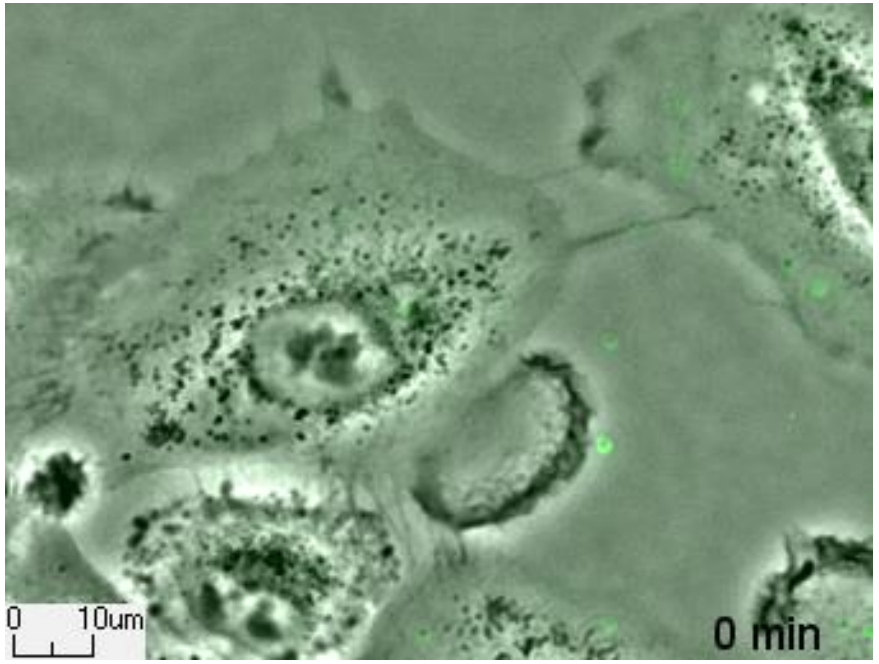
Higher uptake of positively charged silica NPs



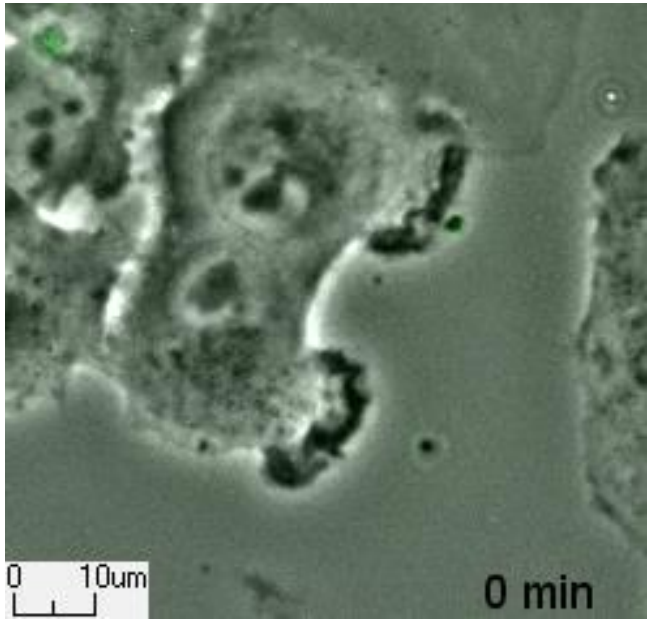
Flow cytometry. NP concentration: 20 µg/ml
a: Statistically significant result with P<0.01 relative to control cells and cells exposed to negatively charged silica NPs.
b: Statistically significant result with P<0.05 relative to control cells.

Detailed uptake information from time-lapse imaging

NP concentration: 20 µg/ml
1 image/minute



A549 cells and positively charged silica NPs

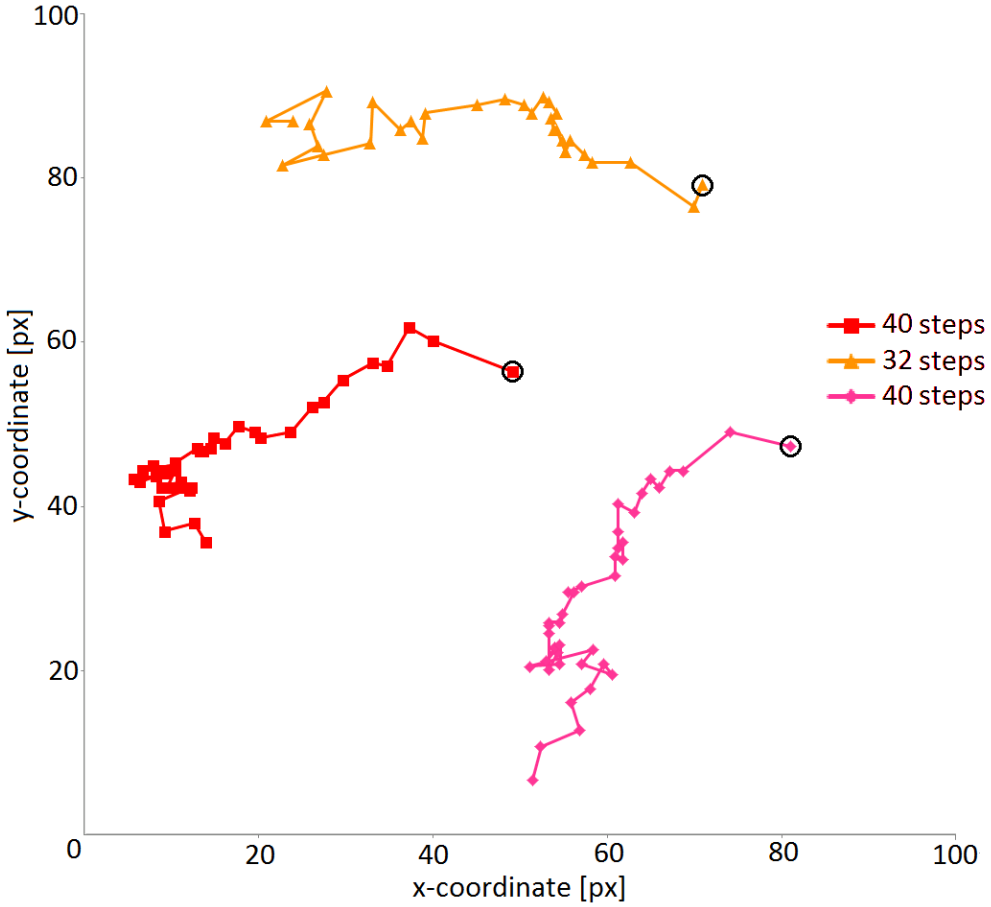


A549 cells and negatively charged silica NPs

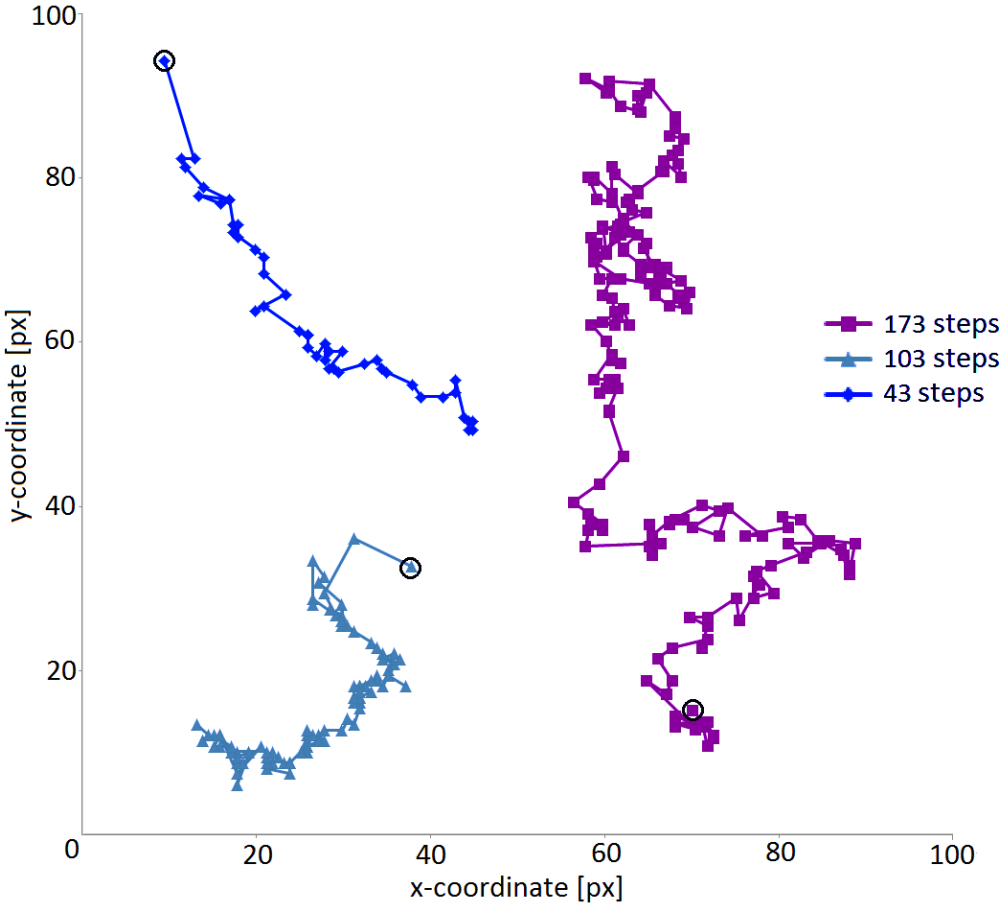
How the silica NPs move when in contact with cells

1 minute/step

Each curve represents one uptake event



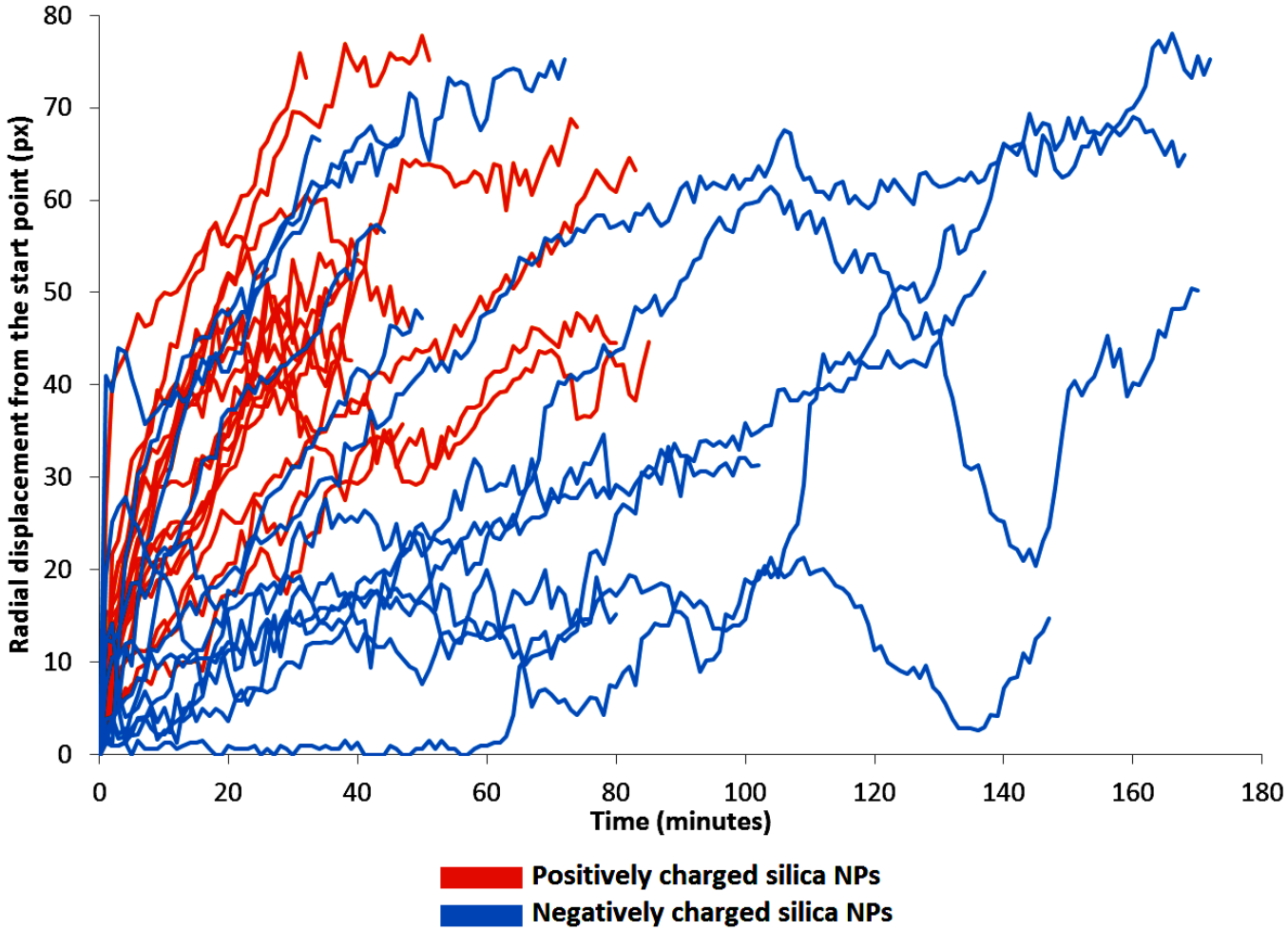
Positively charged silica NPs



Negatively charged silica NPs

The distance between the NPs and the plasma membrane

1 minute/step
Each curve represents one uptake event



Conclusions

Nanoparticles:

- Both the positively and negatively charged amorphous silica NPs were spherical, with high dispersion stability, and a hydrodynamic diameter of approximately 165 nm and 150 nm, respectively.

Toxicity:

- The silica NPs induced no significant reduction in cell viability at 20 µg/ml, and no significant DNA damage, DNA oxidation or induced mutations at 1-300 µg/ml.
- The non-cytotoxic results should be validated at additional concentrations.

Cellular uptake:

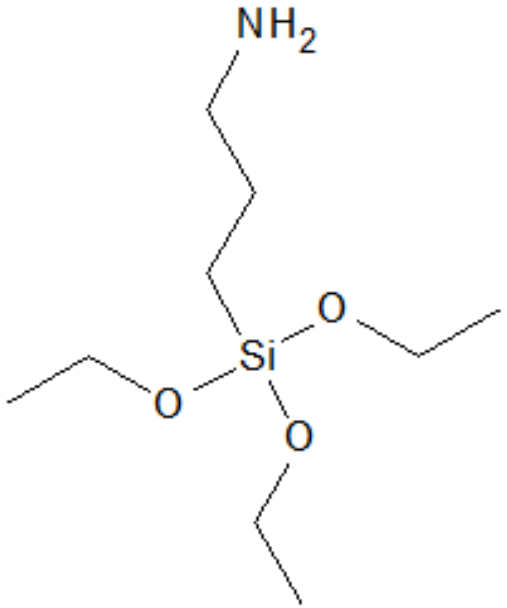
- Both positively and negatively charged NPs were internalized by the cells.
- The positively charged silica NPs were taken up faster and by more cells, compared to the negatively charged silica NPs.

Supplementary figures

Surface functionalization molecules

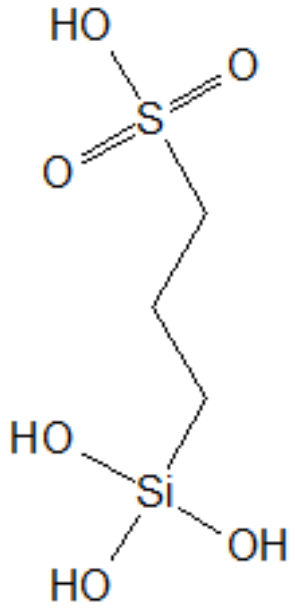
Positively charged silica NPs

(3-Aminopropyl)triethoxysilane (APTES)



Negatively charged silica NPs

3-(trihydroxysilyl)-1-propanesulfonic acid (SIT)



Hydrodynamic diameter of particles in cell culture medium (DLS measurements)