Application Note # 156

Nanoparticle Uptake by Cells Measured Using MP-SPR

Nanoparticles (NPs) are extensively studied as drug delivery systems. NPs enter the cells usually by active transport, i.e. endocytosis. Multi-Parametric Surface Plasmon Resonance (MP-SPR) is previously used for protein-protein interactions is now used in pioneering NP – living cell studies.

Confluent monolayers of human epithelial cervical cancer cells (HeLa) were grown on sensor slides. Uptake of mesoporous silica nanoparticles (SiNPs), branched polyethyleneimine–DNA polyplexes (bPEI–DNA PPs), and extracellular vesicles (EVs) were studied using MP-SPR. Uptake was measured at different temperatures and the activation energy of the cell uptake was calculated using Arrhenius plots.

Introduction

Conventional *in vitro* methods to study uptake of NPs by cells often include labeling of the nanoparticle or the cells. Labeling may affect surface properties of the nanoparticle or behavior of the cells, thus, label-free measurements are desired. Other label-free methods exist; but, MP-SPR is preferred due to its high sensitivity, comprehensive results, temperature range and low sample consumption.

Surface Plasmon Resonance (SPR) is a well-established method to measure binding affinity and kinetics. Powerful Multi-Parametric Surface Plasmon Resonance (MP-SPR) instruments can perform measurements in a wider angular range (40-78 degrees) and at more than one wavelength, extending applicability of SPR also to the study of nanoparticles and cells.

During measurements on cell monolayers, the MP-SPR response is caused by morphological changes and rearrangement of the intracellular material (dynamic mass redistribution) of the cell induced by NP endocytosis.

MP-SPR substrates allow easy *ex situ* growth of cell monolayer on a substrate. Oil-free index matching prevents contamination in the cell experiments. A confluent cell layer is essential for the interaction measurement, preventing any unspecific binding on the substrate. Viability of the cells can be confirmed during the measurement by monitoring full MP-SPR curves and after the measurement *ex situ* using an optical microscope.

Materials and methods

Hydrodynamic diameter of the NPs was determined by dynamic light scattering (DLS). Uptake of NPs in human epithelial cervical cancer cells (HeLa) was measured (Figure 1). Studied NPs were mesoporous silica nanoparticles (P-SiNPs, 154 nm), polyethyleneglycol – polyethyleneimine (PEG–PEI) coated SiNPs (C-SiNPs, 118 nm), branched polyethylenimine–DNA polyplexes (bPEI–DNA PPs, 245 nm), and extracellular vesicles extracted from red blood cells (EVs, 279 nm) (Suutari et al. 2016).

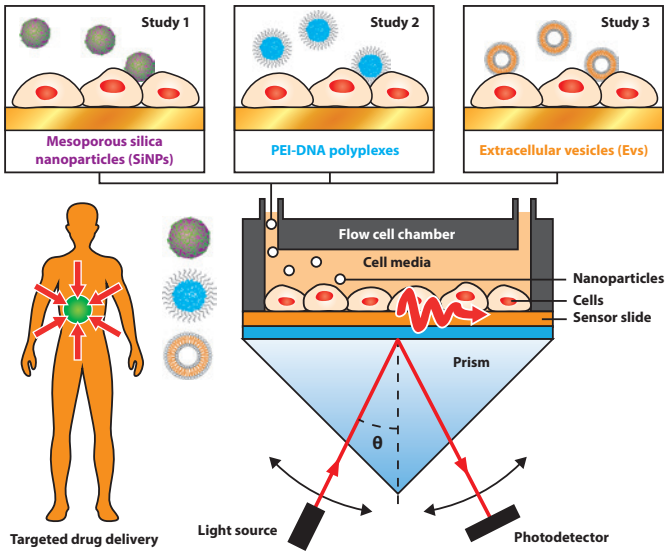

Figure 1. Nanoparticles are used to enhance drug targeting and reduce toxicity of therapy. Cell uptake of mesoporous silica nanoparticles (SiNPs), branched polyethyleneimine–DNA polyplexes (bPEI–DNA PPs), and extracellular vesicles (EVs) were studied using MP-SPR.

Fibronectin was used as an adhesion promoter to grow HeLa cells on gold surfaces *ex situ*. Sensors were inspected with optical microscopy before and after each experiment confirming a confluent and viable cell monolayer (Figure 2).

Nanoparticles (10 µg/mL) were injected and the response of the cells was monitored using MP-SPR Navi™ 200-L OTSO instrument. The rate constants of endocytosis at different temperatures were determined.

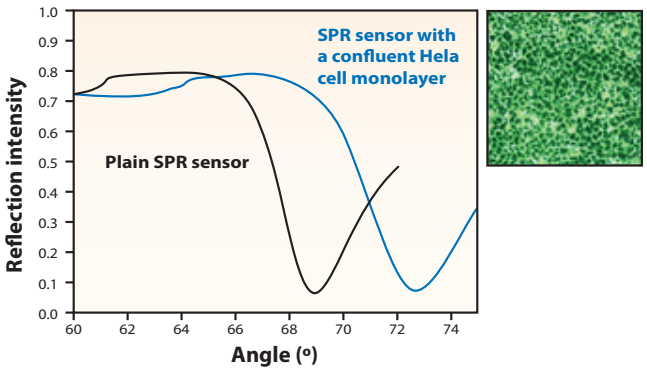

Figure 2. Full SPR curve of gold sensor slide and slide with HeLa cell monolayer. Optical microscopy picture showing the morphology of the confluent HeLa cell monolayer on an SPR sensor.

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Results and discussion

MP-SPR is able to monitor cell uptake of nanoparticle in real-time and to distinguish cell uptake efficacy of different NPs. Uptake of NPs is occurring from the apical side of the cells when a confluent monolayer is attached to the substrate from the basolateral side (Figure 2).

Study1: Uptake of positively charged C-SiNPs nanoparticles in HeLa cells was more efficient and caused a larger response in MP-SPR than uptake of negatively charged P-SiNPs (Figure 3). HeLa cells have a negative resting potential causing more effective uptake of positively charged NPs than negatively charged vehicles. The obtained results were in good agreement with the confocal microscopy images.

Study2: Uptake of positively charged bPEI–DNA PPs caused much higher MP-SPR responses than uptake of silica nanoparticles. This was caused by stronger positive charge and thus more effective uptake of NPs. Uptake of bPEI–DNA PPs were temperature dependent and the uptake decreased when the temperature was increased (20, 28.5 and 37 °C) (Figure 4). The rate constant of bPEI–DNA PPs uptake was 0.039 min^{-1} at 20 °C and 0.232 min^{-1} at 37 °C. These uptake rate constants indicate that bPEI-DNA PPs are taken up by HeLa cells using clathrin-mediated endocytosis or other similar energy dependent pathway.

Study3: EVs uptake by HeLa cells at 37 °C was studied using different doses of EVs. Clear correlation between the response and the EV concentration was detected (Figure 5).

Conclusions

MP-SPR measures interactions of nanoparticles with target molecules, lipid bilayers, biomaterials, and living cells in real-time and label-free. MP-SPR results of nanoparticle uptake by cells were in good agreement with the complementary techniques, such as confocal microscopy.

Baghirov et al. (2016) used MP-SPR to study uptakes of rod-shaped and spherical SiNPs in Madin-Darby canine kidney epithelial cells (MDCKII). MDCKII was used as a model cell line for blood-brain barrier.

See also how drug delivery nanoparticles interactions with target molecules and 100% serum was measured using MP-SPR (AN#152).

Original publication

Suutari et al. Small, 2016,
DOI: 10.1002/smll.201601815

References

Baghirov et al. PlosOne, 2016, 11(8), e0160705

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 200 OTSO, 210A VASA or 220A NAALI

Sensor surfaces: Au, other metal or inorganic coating

Software: MP-SPR Navi™ Control, DataViewer and TraceDrawer™ for SPR Navi™

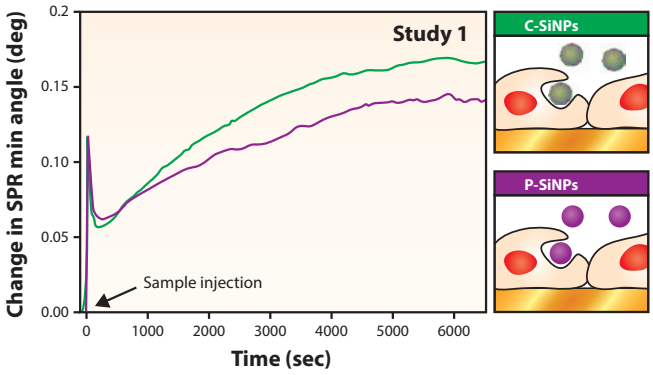

Figure 3. Uptake of mesoporous silica nanoparticles in human epithelial cervical cancer cells (HeLa). Positively charged nanoparticles (C-SiNPs) showed a more efficient uptake and caused a larger response in MP-SPR than negatively charged NPs (P-SiNPs). There was a rapid initial response when SiNPs reached the flow-cell, due to some remaining stock solvent dimethyl sulfoxide (DMSO) after dilutions.

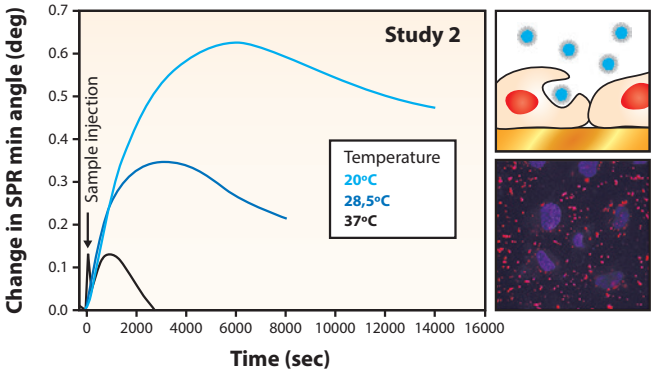

Figure 4. Uptake of branched polyethyleneimine–DNA polyplexes (bPEI–DNA PPs) in HeLa cells in three temperatures. The uptake decreased when temperature was increased. Confocal microscopy images were used for reference. The picture was taken after 4 hours incubation at 37˚C.

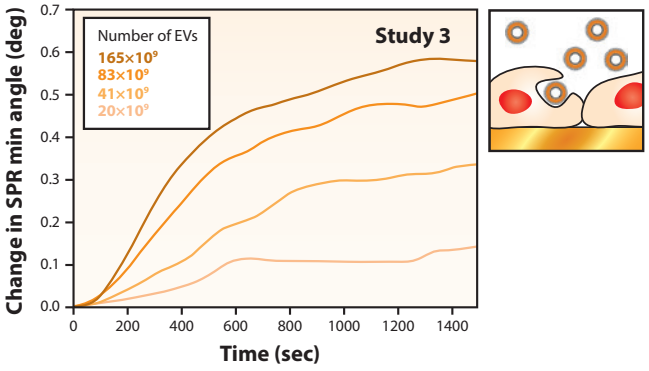

Figure 5. Extracellular vesicles (EV) uptake by HeLa cells was concentration dependent.

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