**Cellular effects of silver nanoparticles or ions? A new method to realize the paradigm-shift**

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Silver nanoparticles (Ag NPs; diameter < 100nm) are the most widespread commercially-used nanoparticles to date, due to their anti-bacterial effects. Controversially, toxicologists point out that the interaction of Ag NPs with cells and the involved pathways of uptake and consequent cellular fate are poorly understood and might pose possible adverse effects. The basis for discrepancy lays partially in the complex physico-chemical properties of the particles: Ag NPs might aggregate or oxidize into silver ions (Ag+) in aqueous environments (such as lakes, rivers, but also blood, extracellular space, cytoplasm, etc.). Aggregation of Ag NPs reduces the surface facing the environment and substantially influences the rate of oxidation. Hence, aggregation changes the way the cell perceives the presence of Ag considerably.

The study of uptake and cellular fate is aggravated by technical limitations in Ag NP visualization, especially in situ (inside cells or tissue). Ag NPs cannot be resolved by bright-field light microscopy; the resolving limit of light is about 250 nm). Sufficiently high resolution can be achieved by transmission electron microscopy (TEM) but the unambiguous detection of Ag NPs remains challenging. Alternatively, indirect methods such as inductively coupled plasma mass spectrometry (ICP-MS) provide information about the Ag content associated with an entire cell culture, but cannot discriminate between intracellular and extracellular/associated Ag. Furthermore, the aggregation state of Ag NPs cannot be retrieved by any of these approaches. Thus, the relative influence of aggregation of Ag NPs and the relative effect of ionic Ag remains an active research topic.

Here, we propose to introduce hyperspectral imaging (HSI) to retrieve this vital - but hitherto unavailable - information on the uptake and intracellular kinetics of Ag NPs. The absorbance of specific wavelengths in the infrared and visible wavelength region is described at interfaces between metals and a dielectric medium, such as water. The effect is known as surface plasmon resonance (SPR) and is absent in non-metals. Hence, Ag NPs can be directly detected in cellular environments. HSI is a newly emerging technique in light microscopy that allows the recording of an absorbance spectrum for each pixel in the image. The absorbance peak is element dependent (*i.e.* an elemental fingerprint) and therefore it is possible to identify with high confidence the presence of Ag. Furthermore, the SPR of Ag NPs undergoes a red shift when particles aggregate, allowing quantification of the aggregation state of Ag NPs.

The quantification of intracellular Ag NPs, including their aggregation state, can be directly correlated to toxicological tests, which promises a paradigm-shift in the understanding of Ag NPs on the cell. Additionally, since Ag+ ions originate from Ag NPs, the relative abundance of intracellular ionic Ag can be evaluated and related to the toxicological data. The HSI approach can be easily extended for experiments that focus on trafficking (to show in which cellular compartment the Ag NPs ends up) and time-lapse data, revealing the kinetics of Ag NP exposure.