Combination of SERS and advanced microscopic methods for the investigation of protein-particle-cell interactions

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Studies on the interaction of nanomaterials with cells have been gaining much interest, especially in the context of nanosafety, but also of the utilization of nanostructures for medical applications, e.g., as nanosensors for use in cells. Regarding both aspects, cytotoxicity and bionanosensing, silver and gold nanoparticles represent an interesting system for basic studies, as their cytotoxic properties and capability to act as elements of optical sensors functioning, e.g., based on surface-enhanced Raman scattering (SERS) are known. The primary interaction between nanomaterials and living organisms is mediated by the particle surface in the biological medium. Thus, the thorough investigation of chemical and physical properties of nanoparticles and the processes from uptake to potential apoptosis are very important for the understanding and evaluation of nanoparticle cytotoxicity.

In the experiments reported here, nanoparticles are transferred into eukaryotic cells from the cell culture medium via fluid phase uptake. A combination of different techniques were utilized to study i) the intracellular distribution and agglomeration behaviour, ii) the molecular composition of the immediate vicinity, and iii) the cytotoxicity of gold and silver nanoparticles. (1). Prior to the *in vitro* tests in the cells, stability of silver nanoparticle suspensions in cell culture relevant media was evaluated by UV-vis, DLS and TEM, revealing the formation of a protein corona around the particles. SERS enables spatially resolved investigation of the uptake and transport mechanism of silver nanoparticles inside living cells and gives chemical information about their local environment, since the nanostructures act as plasmonic substrate. To complement the SERS data, the cytotoxic behaviour of the nanoparticles was determined by XTT assay.

The interaction of the nanoparticles with extra- and intracellular molecules was studied using a combination of Raman scattering, TEM, and synchrotron X-ray microscopy. While the latter two provide information about the localization of the nanoparticles, and their interaction with cellular ultrastructure, we use SERS to learn about the molecular composition of the cellular ultrastructure and the nanoparticle surface. In this way we are able to get insight into the biological response pathways of nanoparticles directly in the living cells, starting from endocytosis, over vesicular transport, to accumulation and apoptosis. The findings may have implications also for other nanomaterials and can improve our knowledge of particle-protein and particle-cell interactions in general.

References

[1] D. Drescher, T. Büchner, A. Matschulat, G. Laube, P. Guttmann, S. Werner, G. Schneider, and J. Kneipp, *submitted.*