Quantification of Nanoparticle Uptake and their Colocalization with cell constituents at single cell level

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The development of nanotechnology in recent years is unprecedented. Understanding the effects of nanoparticles (NPs) on human health is thus of major importance.[1,2] The degree and the mechanism of uptake, localization and distribution of NPs in cells are major issues concerning toxicity and risk assessment, and the effectivity of NPs as delivery devices. A better understanding of the potential effects of nanomaterials on human health is crucial regarding the introduction of nanoproducts into the market and has thus considerable economical importance.

Ion Beam Microscopy (IBM) and Confocal Raman Microspectroscopy (CRM) were therefore employed as label-free techniques capable of detecting and characterizing nanomaterials within single cells. The uptake, intracellular distribution and toxicity of carbon nanotubes (CNTs) and metal oxide nanoparticles in hepatocarcinoma (HepG2) and lung cells (A549) were studied employing these techniques.

By means of IBM the intracellular concentration and distribution of NPs can be established. Overlapping of cell basis element P with CeO_2 NPs can be easily seen in Figure 1. The concentrations of NPs in / or on the cell were calculated in one cell by choosing the mask of cell area. This provides the basis for intracellular dose dependent toxicity studies.

By means of Raman spectra deconvolution and subsequent cross-correlation analysis the colocalization of NPs with different intracellular environments, such as lipid rich regions, cytoplasm and nucleus was quantified. Figure 2 demonstrates the distribution of lipids and poly-(sulfo propyl methacrylate) (PSPM) modified CNTs in HepG2 cell. CRM, furthermore, was capable of detecting nanomaterial induced changes in the secondary nuclear protein structure and nucleobases content. These changes can be used as an indicator of the toxic effect of NPs. This was confirmed with cell proliferation tests. Studies with NPs surface engineered with lipids and polyelectrolytes showed that the nature of the surface of NPs and their modification in biological fluids is crucial for uptake and toxicity.

References

[1] Lee J, Lilly GD, Doty RC, Podsiadlo P, Kotov NA 2009 *Small* **5** 1213-21.
[2] Hillegass JM, Shukla A, Lathrop SA, MacPherson MB, Fukagawa NK, Mossman BT 2010 *Wiley Interdiscip Rev Nanomed Nanobiotechnol* **2** 219-31.
[3] Romero G, Estrela-Lopis I, Zhou J, Rojas E, Franco A, Espinel CS, Fernandez AG, Gao C, Donath E, Moya SE 2010 *Biomacromolecules* **11** 2993-2999

Figures

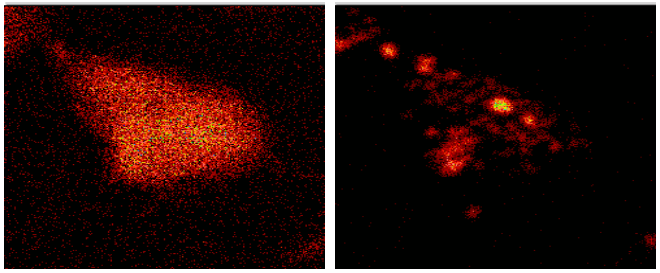

Figure 1. *Proton Induced X-Ray Emission elemental mapping of the cells A549 treated during 72 h with CeO_2 NPs. Left image demonstrate the P distribution and right image – Cerium.*

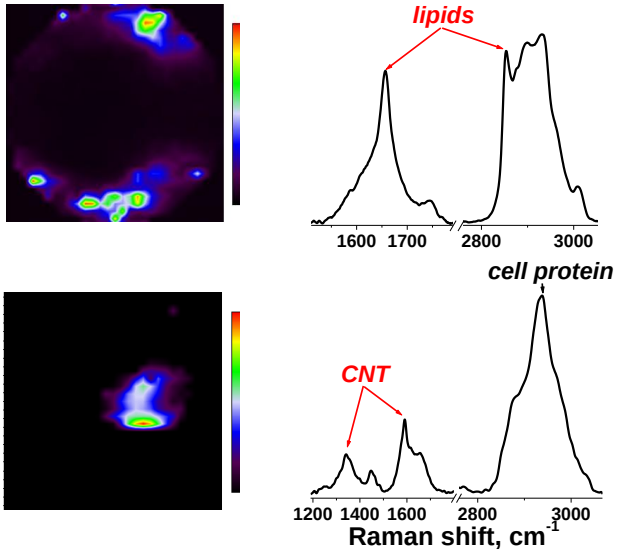

Figure 2. *CRM mappings of a HepG2 cell treated with CNT/PSPM NPs. Top and bottom images show distribution of lipid rich region and CNTs, respectively. The spectra refer to the spot of maximum concentration of both components in corresponding images of the cell.*

Figures

Figure caption