Studying the uptake of nanoparticles by cells to evaluate their potential toxicity

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The astonishing physical and chemical properties of engineered nanomaterials have provoked an exponential growth of nano-products on the free market. Growing concerns over the impact of such materials on human health and the environment have initiated first in depth studies on the effect of nanomaterial exposure to biological systems that showed a high mobility of such materials in organisms or cells [1,2]. In systematic studies, the uptake and the toxic effects of different classes of nanomaterials was compared.

Metallic nanoparticles show strong benefits for separation problems or in medical diagnostics. We thus studied the uptake and the acute cytotoxicity of cobalt and copper containing nanoparticles *in vitro* using two different cell lines (Hela cells and Chinese Hamster Ovary (CHO) cells). Metallic copper nanoparticles were compared to copper oxide nanoparticles and the same amount of copper chloride ions. While a correlation between *in vitro* and *in vivo* data is always difficult, the proposed cell based assays may serve as early indicators for a risk evaluation of these nanomaterials. The role of nanoparticle solubility and surface charge illustrates how physical properties are influencing *in vitro* the behavior of nanoparticles.

For testing the nanoparticle uptake, cell media were mixed with aqueous suspensions of the nanoparticles. After different exposure times, the cells were washed thoroughly and dried in air. After fixation and embedding in a resin (epon), thin sections were cut at room temperature with an ultramicrotome. The slices were mounted on Cu grids, stained with uranyl acetate and lead citrate and finally coated on both sides with a carbon film (ca. 2 nm). The samples were investigated in a Tecnai F30 microscope (FEI, FEG, 300 kV) by scanning transmission electron microscopy with a high-angle annular dark field detector (HAADF-STEM).

Although conventional TEM images of inorganic materials in cell gives clear information about the elemental distribution, e.g. for CeO_2 [3], the contrast in HAADF-STEM (Z contrast) images is improved by the high difference in the atomic number Z [4]. By this technique, the incorporation of CoC and CuC particles in cells could be unambiguously shown. Figure 1a shows two agglomerates of CuC nanoparticles inside a CHO cell. A detailed view on the CoC nanoparticles is represented in figure 1b. The spherical morphology of the embedded material unambiguously proves that these are indeed CuC nanoparticles. In other cases, local enrichments of the staining material (e.g. $PbCl_2$, uranyl acetate) may lead to a similar or even brighter contrast. A misinterpretation of such preparation artifacts can easily be avoided by an EDXS analysis of the respective areas (figure 2).

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1. G. Oberdörster et al., Environ. Health Perspect. **113** (2005) p823.
2. N. Lewinski et al., Small **4** (2008) p26.
3. L. K. Limbach et al., Environ. Sci. Technol. **39** (2005) p9370.
4. L. K. Limbach et al., Environ. Sci. Technol. **42** (2008) p5828.
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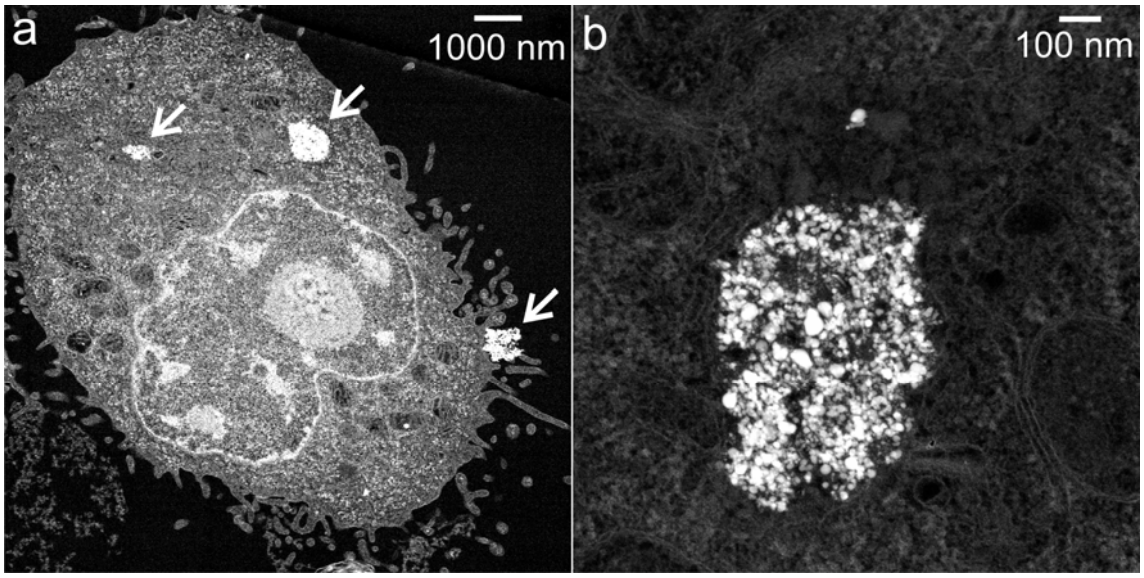

Figure 1. Typical HAADF-STEM images of CuC particles in CHO cells. In (a), two of three CuC agglomerates (marked by arrows) are clearly incorporated inside the cell. (b) magnified view of uptaken CoC particles.

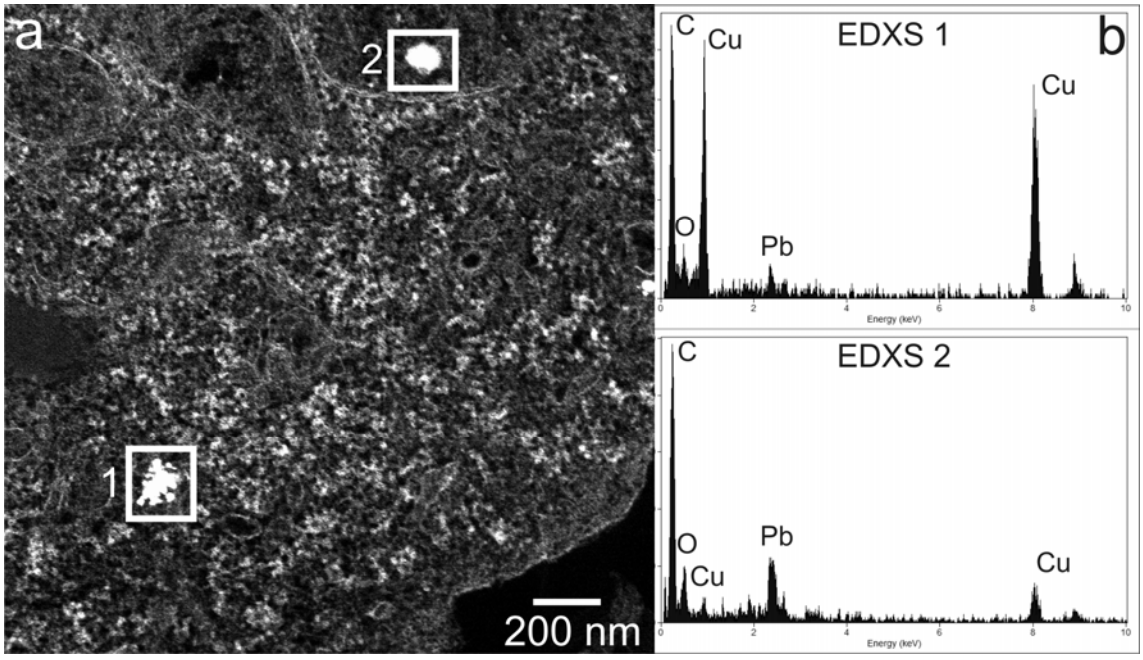

Figure 2. (a) HAADF-STEM images of CuC particles in CHO cells. (b) EDX spectra of the areas marked in (a); area 1 contains mainly CoC, area 2 the Pb-containing staining agent.

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