Bericht zur Forschungsarbeit MBFST-Kennziffer 2818

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„Struktur-Eigenschafts-Beziehungen ausgewählter Nanomaterialien“

„Structure Properties Relationships of Selected Nanomaterials“

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Over the last decade, newly synthesized nanomaterials (NMs) have been found to exhibit fascinating physicochemical properties (size, shape, and composition, surface) that allow them to easily translocate cell membranes, efficiently bind molecular species, and catalyze chemical reactions[1]. In accordance with their enhanced properties, NM has been proposed for use in the fabrication of various devices such as drug and gene delivery, biosensors, virus inhibitors, and protein immobilizers. However, massive quantities of NM would need to be produced for these applications. These developments might increase the potential risk of human exposure and raise additional concern about their short and long-term toxicological effects[2]. In few years, the first integrated nanosystems, functioning much like a mammalian cell systems are expected to evolve[3]. Therefore, a necessary step in assessing NM safety is to establish reliable sources of NM, define and accurately characterize NM properties, and understand the significant interactions with relevant biological systems[4]. However, prior to NM introduction into cellular media for nanotoxicity studies, the development of the synthetic techniques, account for variations in their properties, quality control procedures and toxicity must be addressed[5]. Many biological processes take place at the nanoscale level and these particles interact with bio-molecules exhibiting cellular processes that are critical to life. Therefore, the establishment of nanotechnology using safe-by-design strategy requires the development of assessment tools to identify hazardous nanomaterial properties that could be tailored to improve nanomaterial safety[6].

With the goal of developing broad-based model of environmental toxicology and its injury mechanism at the biological level, a physical library of binary (ZnO, TiO_2, CeO_2) has been developed using versatile Flame Spray pyrolysis (FSP) technique[7]. Variety of solution based precursors such as zinc napthenate (0.5M by metal), Titanium isopropoxide (0.5 M by metal), or Cerium (III) 2-ethylhexanoate (0.5 M by metal) have been used for the syntheses of ultrafine (ZnO, CeO_2 and TiO_2) oxides. The characteristics and the subsequent performance of flame-made particles are affected by precursor composition and oxidant/fuel flow rates. During FSP, a flow of 5 mL/min of liquid precursor delivering to the nozzle using a syringe pump followed by atomising the precursor solution using 5 L/min of dispersant O_2 maintaining a pressure drop of 1.5 bar at the nozzle tip were standard parameters required to obtain the correct particle size. Combustion of the dispersed droplets by the surrounding supporting CH_4 and O_2 (1.5 L/min, 3.2 L/min) formed a source to ignite and sustain spray combustion.

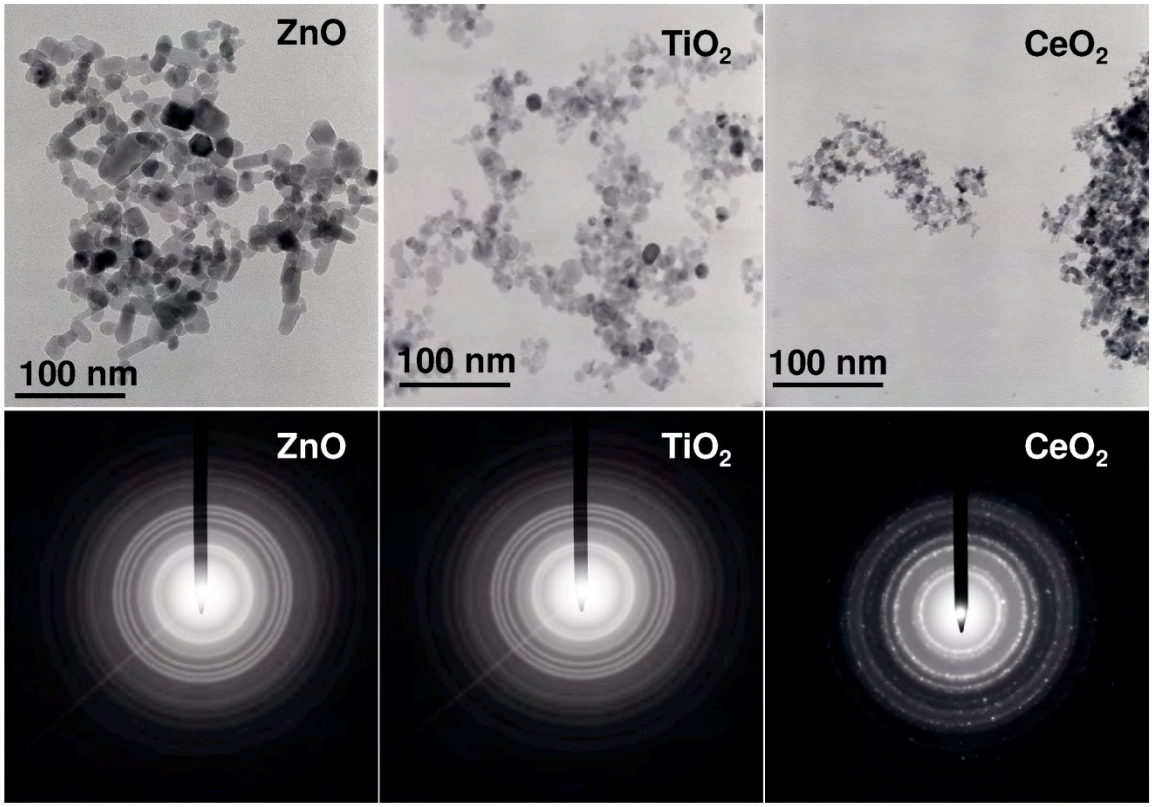

Figure 1. Low resolution TEM of ZnO, TiO_2 and CeO_2 NMs used for the cellular toxicity evaluation

The metal oxide NMs have been analysed using advanced physico-chemical techniques such as Brunauer-Emmet-Teller (BET), X-ray diffraction (XRD), Transmission electron Microscopy (TEM), High resolution transmission electron microscopy (HRTEM), and selected area diffraction patterns (SAED). All the X-ray diffraction patterns of ZnO nanomaterials have been indexed to hexagonal, wurtzite phase[8] (JCPDS No. 36-1451, a = b = 3.2498 Å, c = 5.2066 Å, Z = 2, space group: *P63mc*). TiO_2 crystallites[9-10] was predominately anatase with rutile in the approximate ratio of 4:1. The X-ray diffraction patterns (101), (004), (200), (105), (204) are attributed to anatase while those at (110) and (101) represents rutile phase. FSP synthesis of CeO_2 from a cerium-2-ethylhexanoate precursor yielded plates-like particles of size 5-8 nm. The X-ray patterns at (111), (200), (220), (311), (222), (400), (311), (420), and (422) are indexed[11] to the cubic FCC structure with *Fm*3*m* space group having a lattice constant of 0.5410 nm according to JCPDS 78-0694. The BET surface area and porosity measurements of these oxide materials were performed using adsorption technique. The particle sizes of ZnO, TiO_2 and CeO_2 using BET (d_{BET}) was found to be 18, 10 and 5 nm respectively. The crystallite size (d_{XRD}) was found to agree closely with the particle size obtained from BET. The TEM images (see Figure 1) confirm the crystalline morphology of the binary oxide particles. ZnO, TiO_2 and CeO_2 had short-rod (20 nm), spherical (10 nm) and

plates-like (5 nm) morphology. The highly crystalline nature of these particles was evident from the distinct crystallographic ring patterns in SAED (see Figure 1). The Fourier Filtered HRTEM images (Figure 2) revealed highly resolved power spectra with clear crystallographic arrangements of the particles.

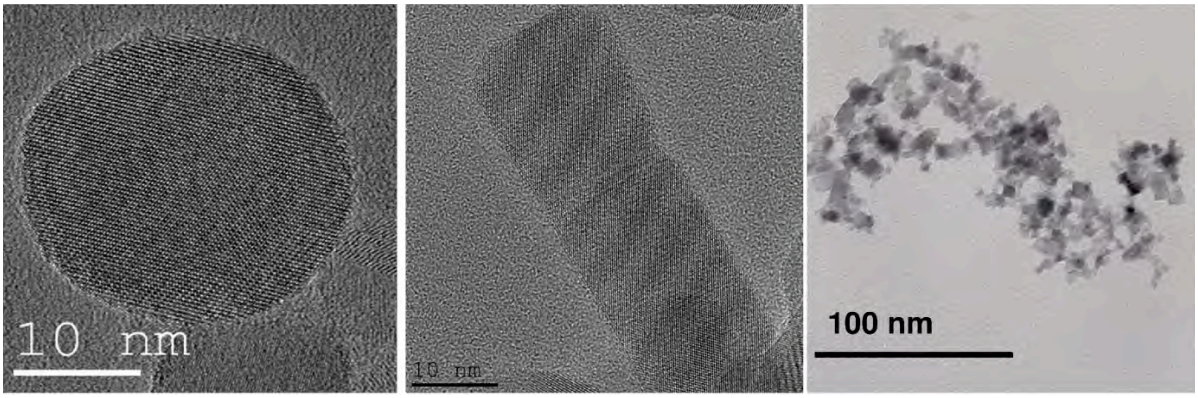

Figure 2. High resolution images of spherical TiO_2, short-rod ZnO and plates-like CeO_2 used for the cellular toxicity evaluation

The toxicity evaluation of the binary metal oxides (ZnO, TiO_2 and CeO_2) in the RAW 264.7 and BEAS-2B cell lines was performed using high-content-screening technique (see Figure 3). Different dye combination (Hoechst 33342, JC1, Hoechst 33342 with Fluo 4, propidium iodide (PI)) was used for rapid dose and time-dependent assessment of nanoparticle toxicity. This work is in collaboration[12] with Prof. Dr. Andre E. Nel, Director, Center of Environmental Implication of Nanotechnology (CEIN), University of California, Los Angeles, USA under cooperative Agreement Number DBI-0830117.

ZnO nanoparticles exerted significant cytotoxic effects due to the generation of ROS as evidenced by the increased intracellular Ca^{2+} flux, lowering of MMP, and loss of membrane integrity collectively known as hierarchical oxidative stress. CeO_2 and TiO_2 did not exhibit any cytotoxic properties. The effect of ZnO on MMP, $[Ca^{2+}]_i$ flux and PI uptake was much pronounced with the 3-6 h exposure of NMs (12.5-25 µg/ml) in RAW 264.7 cells. Interestingly, all of the above responses were observed within 2 h exposure of NMs (6 µg/ml) in BEAS-2B cells. TiO_2 generated small but significant decreases of MMP in both cell types without any effects on cell viability. CeO_2 did not have an effect on any of the response markers. The reason for including different dye combinations (Hoechst 33342 for DNA staining to assess cell number and nuclear size; JC1 to assess mitochondrial membrane potential (MMP); Hoechst 33342 with Fluo 4 to assess the intracellular calcium flux and propidium iodide (PI) to assess increased membrane permeability in dying cells) is the

compatibility of their fluorescent wavelengths and that these are cellular injury responses that capture toxicity information of the metal oxide. In order to develop a rapid screening procedure for ZnO toxicity based on an oxidative stress injury paradigm, we established that the nanoparticle dissolution leads to an increased toxicity (ZnO dissolves in the cell medium where as TiO_2 and CeO_2 does not).

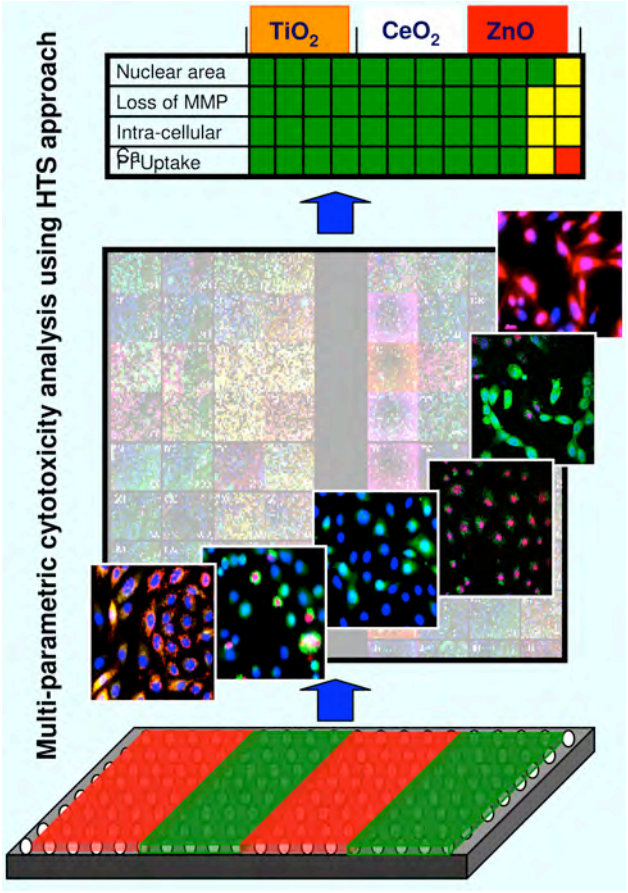

Figure 3. Use of High throughput screening approach for faster identification of potentially toxic nanomaterials (collaboration with Andre E. Nel, CEIN, UCLA, USA)

The dissolution experiments of ZnO nanoparticles in aqueous solution were performed in electrolyte solution followed by aliquot extraction for dissolved zinc analysis. The concentration of dissolved Zn^{2+} released by the undoped nanoparticles rapidly exceeded the saturation zinc concentration for ZnO at pH 7. The dissolution and the toxicity evaluation were performed with a number of fluorescent dyes that was used to assess ROS production and oxidant injury responses. We demonstrated that under non-UV exposure conditions, ZnO NMs induces H_2O_2 and O_2^- production, increased oxidant injury responses and enhanced the loss of MMP, PI uptake and $[Ca^{2+}]_i$ increase in different cell lines.

We demonstrate that high-content screening can be used to speed up nanoparticle hazard ranking according to a toxicological paradigm that can be assessed by a multi-dye

combination that assess nuclear area, MMP, [Ca^{2+}] flux and membrane permeability. The cytotoxicity of ZnO was found to be toxic compared to TiO_2 and CeO_2 nanoparticles in the experimental conditions. This toxicity is explained in terms of dissolution of the ZnO NMs in the cellular medium that is responsible of ROS generation and toxicity parameters.

Presentation of the work:

The findings of the nanoparticle induced toxicity in higher organisms will be discussed (By Prof. Dr. Lutz Mädler) Annual Meeting of Biotechnology in Berlin in September, 2011. The details of the document are as follows:

Lutz Mädler, Demonstration of reduced toxicity in the higher organisms by engineering nanoparticles, 1st European Congress of Applied Biotechnology together with, 29th **DECHEMA**'s Biotechnology Annual Meeting, September 25 - 29, **2011**, ICC Berlin, Germany- **Oral presentation**

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