In vitro toxicity screening of engineered nanoparticles

Gastroenterologische Molekulare Zellbiologie
Med. Klinik und Poliklinik B

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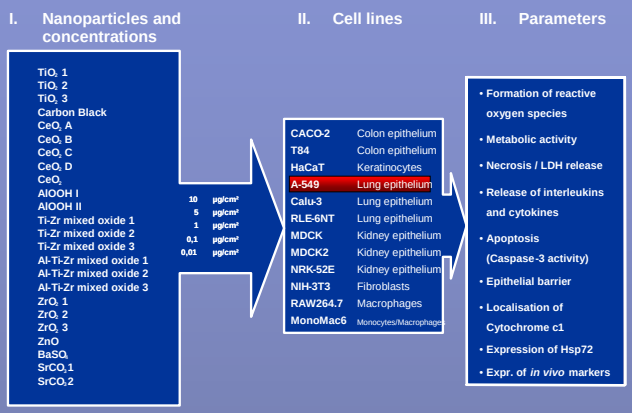
Introduction and Results

We have tested eleven cell lines with respect to three different toxicity parameters (reactive oxygen species formation, metabolic activity, and necrosis) in the presence of 24 different nanoparticles in three to five concentrations. Selected cell lines and particles have also been analyzed regarding the cellular Caspase-3 activity evoked by the particle dispersion applied and the maintenance of the epithelial barrier.



Generation of reactive oxygen species (ROS) was detected in all cell lines after exposure to Carbon Black, however, due to its optical properties, carbon black limits the quantification of ROS with respect to the controls. TiO_2 3 and four CeO_2 (A-D) also triggered the formation of ROS. A mixed oxide of titanium and zirconium (Ti-Zr Mixed Oxide 3) provoked the formation of ROS in six of ten cell lines tested. The metabolic activity of all cell lines exposed was impaired by zinc oxide particles which also triggered the release of lactate dehydrogenase (LDH), an indicator of cell necrosis. Interestingly, the time elapsed after seeding and prior to exposure to ZnO influenced the effect measured. BaSO4 nanoparticles increased the release of LDH in CaLu3 cells while it decreased the metabolic activity in the cell line NIH-3T3. To further characterise the impact of selected nanoparticles *in vitro*, the activity of Caspase-3 and the integrity of the epithelial barrier formed by epithelial cells was investigated in the presence of particle dispersions. The trans-epithelial electrical resistance (TEER), which is a measure for the epithelial barrier, was found to be impaired only by ZnO particles in two kidney epithelium like cell lines. The activity of Caspase-3, which is a key enzyme in apoptosis, was elevated after exposure to ZnO particles and was not increased by Carbon Black, ZrO_2 1, Ti-Zr Mixed Oxide 3, and TiO_2 3 nanoparticles.

Conclusion and Outlook

Overall, only a small fraction of the particles tested in the present screening showed significant effects on the eleven cell lines investigated. The degree of cellular reactions observed showed that it is necessary to expose at least 6 validated cell types to the nanoparticle in question. Sensitive instead of robust cell lines should be used. Furthermore, short term (e. g. oxidative stress), mid-term (e. g. cell death), and long-term (e. g. mutations, transformation) parameters have to be investigated as different particle types may excert effects at different points of time. In terms of assay read-outs, tests requiring optical and chemical detection should be avoided in favour of detections that may not be interfered with by nanoparticles. It is essential to validate each test for each type of nanoparticle.

Results

	ROS	MTT	LDH	Cas3	TEER
TiO_2 1	-	-	-	nd	nd
TiO_2 2	-	-	-	nd	nd
TiO_2 3	+/-	-	-	-	-
Carbon Black	+	-	-	-	-
CeO_2 A	+/-	-	-	nd	nd
CeO_2 B	+/-	-	-	nd	nd
CeO_2 C	+/-	-	-	nd	nd
CeO_2 D	-	-	-	nd	nd
CeO_2	-	-	-	nd	nd
Böhmit I	-	-	-	nd	nd
Böhmit II	-	-	-	nd	nd
Ti-Zr Mixed Oxide 1	-	-	-	nd	nd
Ti-Zr Mixed Oxide 2	-	-	-	nd	nd
Ti-Zr Mixed Oxide 3	+/-	-	-	-	nd
Al-Ti-Zr Mixed Oxide 1	-	-	-	nd	nd
Al-Ti-Zr Mixed Oxide 2	-	-	-	nd	nd
Al-Ti-Zr Mixed Oxide 3	-	-	-	nd	nd
ZrO_2 1	-	-	-	-	-
ZrO_2 2	-	-	-	nd	nd
ZrO_2 3	-	-	-	nd	nd
ZnO	-	+	+	+	+
$BaSO_4$	-	+/-	-	nd	nd
$SrCO_3$ I	-	-	-	nd	nd
$SrCO_3$ II	-	-	-	nd	nd

Table 1: Survey of the results of the in vitro toxicity screening of 24 engineered nanoparticles. ROS: reactive oxygen species (oxidative stress); MTT: reduction of MTT (metabolic activity); LDH: release of lactate dehydrogenase (cell death); Cas3: activity of Caspase-3 (apoptosis); TEER: transepithelial electrical resistance; - no effect detected; + significant effect detected in all cell lines tested; +/- significant effect detected in some of the cell lines tested; nd: not determined.

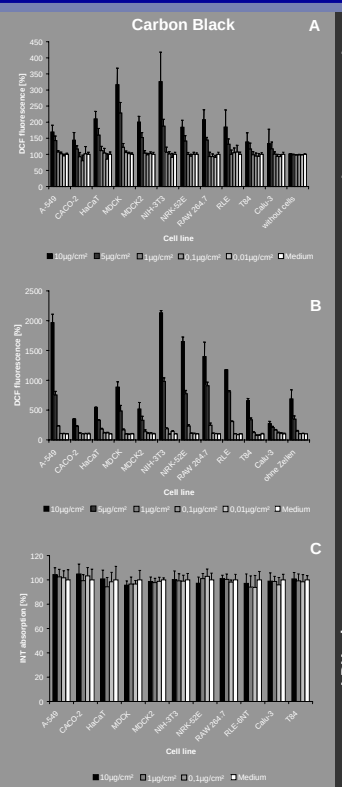


Figure 1: **A** Oxidative stress (expressed as mean DCF fluorescence [%]) after 1h, **B** after 3h; **C** cell death (measured by LDH activity, expressed as mean $INT_{released}$ absorption [%]) in eleven different cell lines exposed to dispersions of Carbon Black or stirred cell culture medium (control). Standard deviations are indicated.

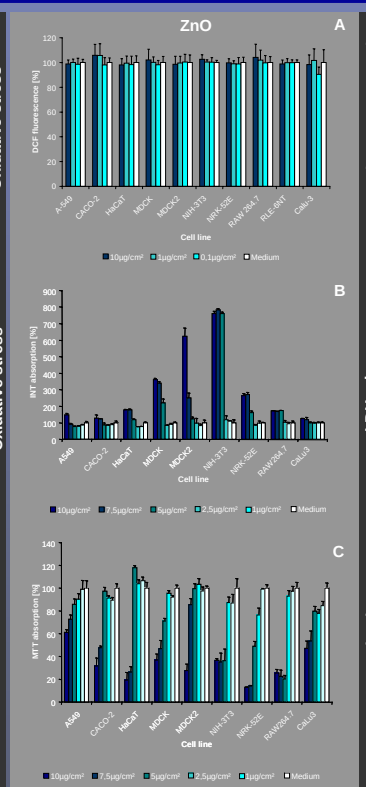


Figure 2: **A** Oxidative stress (expressed as mean DCF fluorescence [%]), **B** metabolic activity (expressed as mean $MTT_{reduced}$ absorption [%]), **C** cell death (measured by LDH activity, expressed as mean $INT_{reduced}$ absorption [%]) in nine resp. ten different cell lines exposed to dispersions of ZnO NP or stirred cell culture medium (control). Standard deviations are indicated.

Methods

All cell lines were ordered from ATCC, HaCaT cells were supplied by CLS, MDCK2 cells were supplied by ECACC. Nanoparticle dilutions as well as pure medium required for positive and negative controls were stirred for 24h at room temperature and 900rpm according to the NanoCare dispersion specifications (BASF 2007). The measurements of ROS formation, MTT reduction, and LDH release were performed according to the respective standard protocols provided on the NanoCare server. The assay kit EnzChek® Caspase-3 by Molecular Probes was utilized to measure the activity of Caspase-3.