ASSESSING INTERFERENCES OF UPCONVERTING NANOPARTICLES WITH *IN VITRO* TOXICITY ASSAYS

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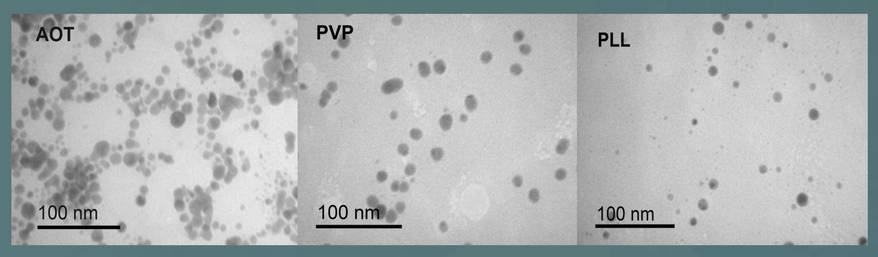
INTRODUCTION

The recognition of upconverting nanoparticles (UCNPs) as promising candidates for the development of novel bio-imaging methods [1] brings about the need for accurate appraisal of their biocompatibility and safety. The primary way of assessing nanoparticle (NP) safety is using *in vitro* assays, which often employ measurements of absorbance or fluorescence [2]. Nanoparticles possess exceptional physicochemical properties (large surface area, high reactivity) that may lead to interferences with assay components and detection systems [3]. Tests with coated iron oxide and silver NPs are known to result in false conclusions about their toxicity, exactly due to interferences [4].

This study examines upconverting, downconverting and silver NPs with the aim of detecting the interferences with commonly used cytotoxicity assays. The assays in question are used to determine metabolic activity (MTT and MTS reduction), oxidative stress (DCFH-DA oxidation) and viability (Neutral Red).



d_H = 15,2 ± 5,3 nm, ζ = -40,6 ± 6,5 mV | d_H = 5,0 ± 1,6 nm, ζ = -18,0 ± 3,7 mV | d_H = 5,4 ± 0,4 nm, ζ = +38,6 ± 6,9 mV

Figure 2. Transmission electron micrographs of differently coated silver NPs. Included below are the respective hydrodynamic diameter (d_H) and zeta potential (ζ).

MATERIALS AND METHODS

Five types of NPs were chosen for this study: upconverting Er0,5Yb10:$BaGdF_5$ (UCNPs), downconverting Eu10:$BaLuF_5$ (DCNPs) and silver NPs coated with three different coatings – negatively charged sodium bis(2-ethylhexyl) sulfosuccinate (AOT), neutral poly(vinylpyrrolidone) (PVP) and positively charged poly-L-lysine (PLL). Four commonly used assays were tested for interferences: MTT and MTS cell viability assays, DCFH-DA oxidative stress assay and Neutral Red cytotoxicity assay. All experiments were performed in a cell-free system. Different concentrations of NPs were added to 96-well plates and treated with each dye according to the manufacturer's instruction. Control measurements were performed without the addition of NPs.

Additionally, two methods were employed to determine the viability of human astrocytes in the presence of UCNPs and DCNPs. The classical MTT assay was compared to flow cytometry analysis, which is unaffected by NP interferences. 1321N1 cells were exposed to three different concentrations of NPs for 24 hours. Both the MTT assay and the flow cytometry analysis were performed according to the manufacturer's protocol. For flow cytometry, the cells were stained with propidium iodide and Annexin V-FITC.

	NP (mg/L)	MTT cell viability (% of control)	Flow cytometry cell viability (% of control)
Control	0	100	99
Er0,5Yb10: BaGdF5	10	99	96
	50	99	98
	100	95	97
Eu10: BaLuF5	10	106	97
	50	100	93
	100	99	92

Table 1. Comparison of the cell viability tests performed on human astrocytoma (1321N1) cells. The results are expressed as the percentage of living cells after exposure to NPs. Results indicate that both UCNPs and DCNPs were not toxic up to 100 mg/L.

CONCLUSION

For all of the NPs, interferences derived from the optical properties of NPs, their adsorptive capacity and ability for chemical interaction with assay components. Results fluctuated depending on metal core and surface coating of NPs, as well as on the assay system. The observed non-biological artefacts claim that a comprehensive characterization of the intrinsic properties of metallic NPs and their stability in biological media should be performed prior to any biological experiments. Indeed, all of the tested NPs exhibited size distributions that rapidly increased upon suspension in cell culture media implying their instability.

In view of existing gaps for a robust biomedical testing of novel NPs, it is imperative that any investigation of biocompatibility and safety of NPs be carefully accomplished bearing in mind every possible interference and interaction with the test system.

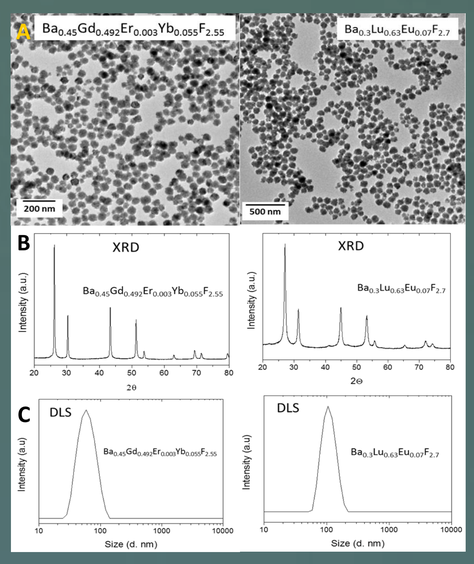


Figure 1. Transmission electron micrographs (A), XRD spectra (B) and DLS spectra (C) of studied upconverting (left) and downconverting (right) NPs.

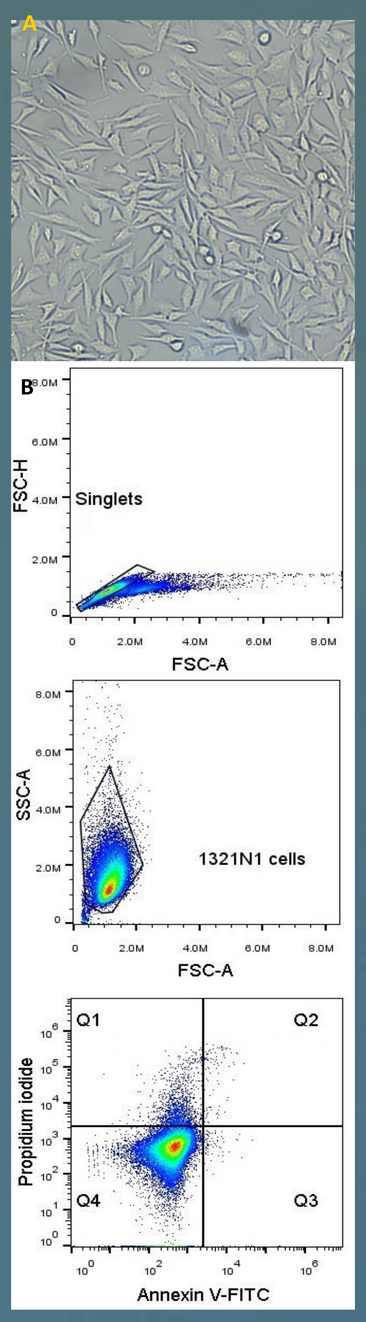


Figure 6. TEM images of human astrocytes (A), and gating strategy for flow cytometry analysis (B). The results show negligible amounts of dead or apoptotic cells.

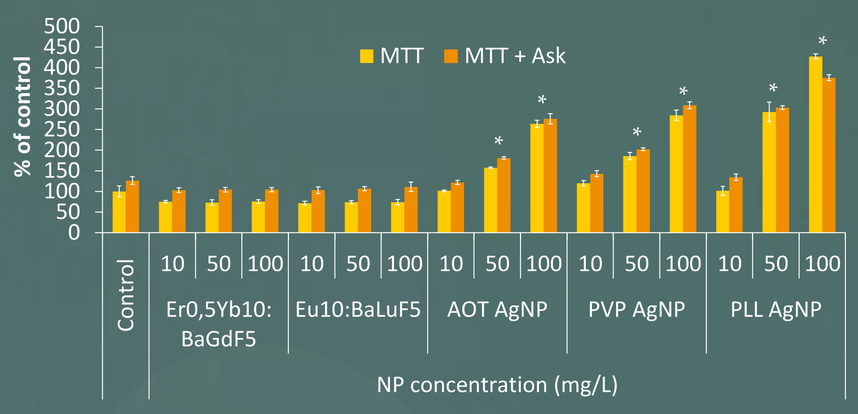


Figure 3. Effects of different concentrations of studied NPs on the MTT cell viability assay. The interference was assessed without (yellow) and with (orange) the addition of ascorbic acid as a reducing agent. Asterisk marks values that are significantly different from the control. UCNPs and DCNPs show no effects, while AgNPs strongly increase the absorbance in higher concentrations.

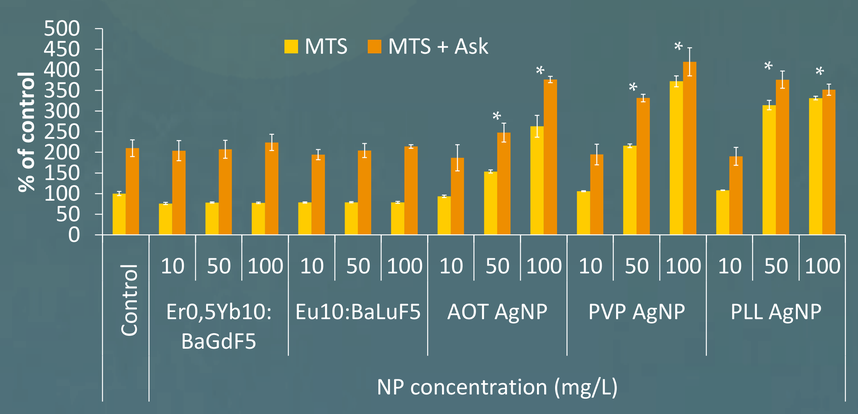


Figure 4. Effects of different concentrations of studied NPs on the MTS cell viability assay. The interference was assessed without (yellow) and with (orange) the addition of ascorbic acid as a reducing agent. Asterisk marks values that are significantly different from the control. Again, there are no interferences from UCNPs and DCNPs, but AgNPs interfere depending on the dose.

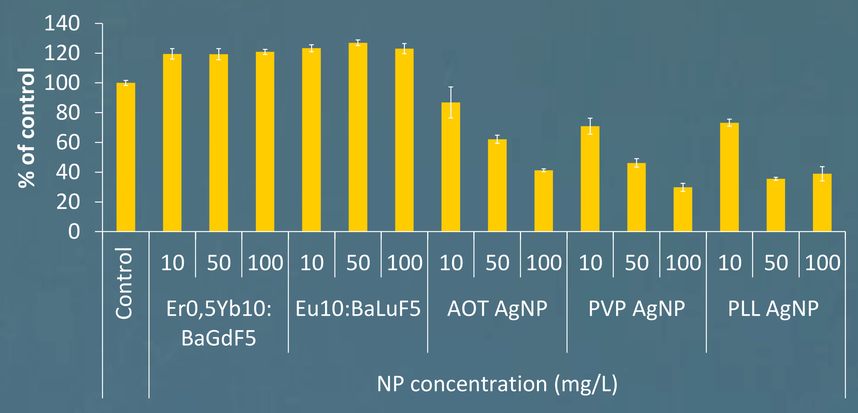


Figure 5. Effects of different concentrations of studied NPs on the DCFH-DA oxidative stress assay. Fluorescence intensity was measured after 60 min of dye incubation. All of the values are significantly different from the control. UCNPs and DCNPs show positive interference independent of the dose. AgNPs demonstrate dose-dependent fluorescence quenching.

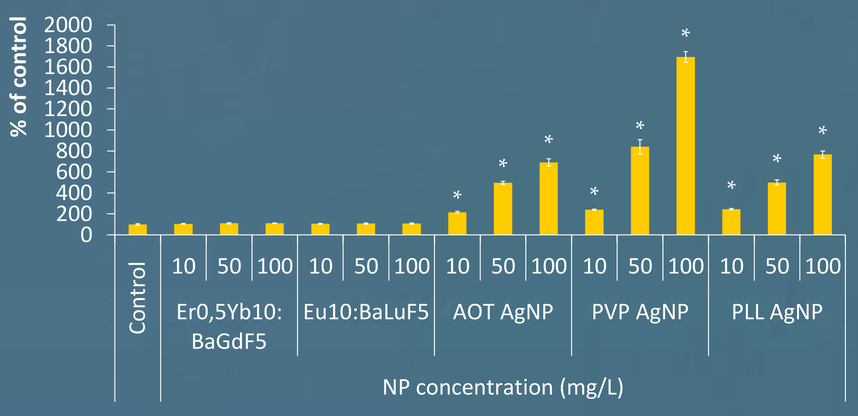


Figure 7. Effects of different concentrations of studied NPs on the Neutral Red cytotoxicity assay. Asterisks mark values that are significantly different from the control. UCNPs and DCNPs do not interfere with the assay, while AgNPs do even in the lowest concentrations.

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