Title *

A comparison of the toxicity of nanomaterials to the HL60 neutrophil-like cell line and primary human neutrophils *in vitro*

Abstract *

The pulmonary hazard of nanomaterials (NMs) is usually assessed *in vivo* by assessing neutrophil accumulation as an indicator of inflammation amplitude and duration. In order to move away from animal testing, relevant in vitro models are required, however, there are a lack of studies which have assessed neutrophil responses to NMs *in vitro.* Using cells of human origin is preferred when screening toxicity *in vitro*, and so this study compared the response of the human HL60 neutrophil-like cell line, with that of primary neutrophils isolated from human blood. A panel of nanomaterials were tested; silver (Ag), zinc oxide (ZnO), copper oxide (CuO), titanium dioxide (TiO_2) and multiwalled carbon nanotubes (MWCNTs), and impacts on cell function were investigated via assessment of; cytotoxicity (alamar blue assay), cytokine production (e.g. GROα. IL-8, MCP-1) and activation of a respiratory burst (cytochrome c assay) at concentrations <125ug/mL and time points < 24h. For the responses measured the response of the cell line was comparable to that of the primary cells, with the exception of ZnO, which was relatively less toxic to primary cells than the HL60 cell line. Overall, the toxicity of NMs was ranked Ag>ZnO>CuO>MWCNTs>TiO_2. Interestingly the ability of these exact nanomaterials to exhibit a pulmonary inflammatory response has been investigated previously *in vivo*, with a similar pattern of toxicity observed to this *in vitro* study. The results suggest that *in vitro*models are useful and can be used to screen the toxicity of NMs, and that their use should be encouraged to better align nanotoxicology testing with the 3Rs principles.

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