

# ***In vitro* cytotoxicity of nanoparticles, accounting for agglomeration & settling**

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Nanoparticles made by aerosol-based technologies find a wide range of promising applications (Pratsinis, 2010), though concerns for their potential health impact exist (Xia *et al*, 2009). Upon *in vitro* cytotoxicity evaluations of such particles, agglomeration and settling need to be considered as they directly influence the particle dose deposited on the employed cell cultures (Limbach *et al*, 2005).

Here, nanoparticles of various chemical compositions and sizes are made by flame spray pyrolysis (Madler *et al*, 2002) and their *in vitro* cytotoxicity against murine macrophages is evaluated (Sotiriou *et al*, 2014), accounting for their agglomeration and settling in cell culture media. The agglomerate size distributions of the particles in suspension are measured by dynamic light scattering (Wengeler *et al*, 2006) and their settling is evaluated by ultraviolet-visible spectroscopy (Sotiriou and Pratsinis, 2010). The cell viability after 24 h incubation with such particle suspensions is monitored by a tetrazolium salt reduction assay in both upright (Sotiriou *et al*, 2014) and inverted cell culture configurations (Cho *et al*, 2011, Lee *et al*, 2014) (Fig. 1).

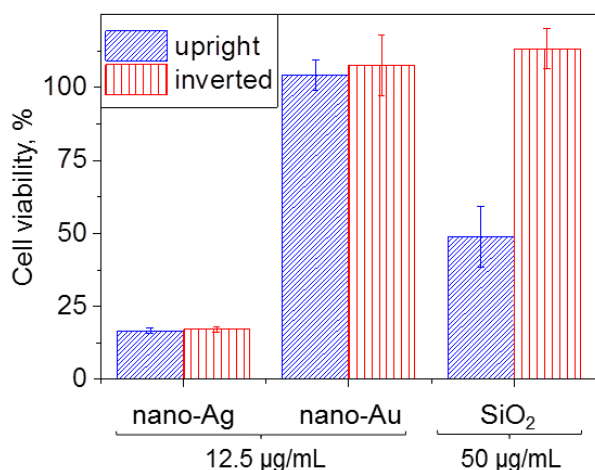


Figure 1. Relative viability of RAW 264.7 murine macrophages after 24 h incubation with nanosilver, nanogold or silica suspensions in standard upright and inverted cell culture configurations.

Three categories of nanoparticles can be distinguished, based on their cytotoxicity. Materials such as nanogold (of 14 nm average crystal size) induce no reduction in cell viability over a wide concentration range (Sotiriou *et al*, 2014) and independent of the spatial configuration of the cells (Fig. 1). Silver particles of relatively small crystal size (~8 nm) induce significant

cytotoxicity in both configurations due to silver ion release from their surface (Sotiriou *et al*, 2014) (Fig. 1). Finally, materials such as agglomerated silica nanoparticles (of 30 nm average primary particle size) induce higher cytotoxicity in upright than in inverted cell culture configuration, due to enhanced particle-cell contact in the former (Fig. 1).

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