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Protein coronas modulate silver nanoparticle sulphidation *in vitro* with impact on cell toxicity

Proteins bind to nanoparticles (NPs) in biological media, forming what is known as protein coronas (PCs), which provide the biological identity of NPs[1] and are thought to impact cell uptake[2]. Silver NPs are among the most widely used nanomaterials and their toxicity has been extensively studied[3]. They have been shown to exhibit lower toxicity when transformed into Ag_2S; this phenomenon was studied predominantly in the absence of protein[4].

We have shown for the first time that PCs play an important role in the sulphidation of Ag NPs *in vitro*, in media relevant for cell toxicity studies[5]. We present a mechanism by which strongly-bound proteins can trap Ag^+, subsequently transformed into nano-Ag_2S crystals due to the presence of small Sulphur-containing molecules in cell culture media. Furthermore, we combine transmission electron microscopy (TEM) with x-ray elemental analysis, atomic absorption spectroscopy and UV-Vis spectroscopy to show that rapidly-exchanging proteins carry Ag^+ away from the particle surface, thus preventing Ag_2S formation, in a protein concentration-dependent manner (Figure 1). We study the impact of both Ag^+ concentration and Ag/S ratios on the formation of nano-Ag_2S.

We show that even partial sulphidation of Ag NPs results in decreased cytotoxicity, while complete sulphidation prevents overproduction of pro-inflammatory cytokines in J774 macrophages.

Given that in general *in vitro* results, obtained at low serum content, are used to predict *in vivo* toxicity of NPs, and that protein concentrations are much higher in living organisms than in cell culture media, the mechanism we present here, where an increase in serum concentration and, hence, in the content of rapidly-exchanging proteins[6] results in decreased Ag_2S formation and promotes toxicity of Ag NPs has important implications regarding toxicity studies on nanomaterials.

References

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Figures

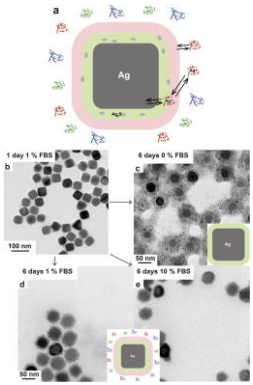

Figure 1: a) Proposed mechanism of Ag NP sulphidation in the presence of PCs (green – long-lived coronas, pink – corona of rapidly-exchanging proteins) and bulk protein b) TEM image of Ag NPs with preformed long-lived coronas after 24 h in cell culture media (CCM) supplemented with 1% foetal bovine serum (FBS) followed by prolonged incubation in serum-depleted CCM, to only preserve the long-lived PC (c) or in CCM supplemented with 1% FBS (d) or 10% FBS (e), to ensure different amounts of rapidly-exchanging proteins, resulting in distinct degrees of Ag NP sulphidation (adapted from 5).