An in vitro strategy to assess mitigation of hazardous properties of engineered metal nanoparticles

Abstract Book

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3.16. An *in vitro* strategy to assess mitigation of hazardous properties of engineered metal nanoparticles

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The huge progress in the nanotechnology field has requested the production of increasingly advanced engineered nanoparticles (NPs). In particular, metal-based advanced NPs are widely used in several industrial applications. However, their potential effects on human health during occupational exposure are still incompletely characterized thus far and possible strategies to decrease their hazardous properties are not yet clearly defined. In this project we are developing an *in vitro* approach to test the cytotoxic effects of metal-based NPs, as derived from production lines or modified through coating with organic or inorganic moieties. We have used two cell models widely employed in toxicological studies, the human alveolar cell line A549 and the murine macrophage cell line RAW264.7, to avoid possible limitations due to cell specific effects. Moreover, in order to evaluate the effectiveness of mitigation approaches for NPs endowed with little acute cytotoxicity, additional endpoints, alternative to viability, have also been assessed. Colloidal suspensions of Ag, TiO_2 and ZrO_2 NPs were tested as provided by industries or modified with SiO_2 NPs or citrate used as coating remediation agents. Heterocoagulation of opposite charged phases was applied in order to promote the coating of pristine surfaces by modifying agents. Heterocoagulated sols were obtained by ball milling sols of positive charged Ag, TiO_2 and ZrO_2 NPs with negative charged SiO_2 NPs or citrate ions. Modified samples, obtained by spray-drying and re-dispersing in water the corresponding sols, were also obtained in order to compare reactivity. Original and modified NPs were added to culture media starting from water colloidal suspensions. Viability was determined with the resazurin method in a range of doses from 2.5 to 80 nfg/cm^2 (0.3125 to 20 μg/cm^2 for Ag NPs) of monolayer surface at three experimental times (24, 48 and 72h). The expression of the inducible form of nitric oxide synthase (*Nos2*), an indicator of macrophage activation and, hence, of pro-inflammatory activity, was assessed with RT-PCR as an end-point alternative to viability.

Among the NPs tested, only Ag NP caused a significant loss of viability, with an IC_{50} of about 0.8 μg/cm^2 for Raw264.7 cells and 2.4 μg/cm^2 for A549 cells at the 24h-experimental time. In a preliminary experiment, SiO_2 NPs were demonstrated to have no significant effect on cell viability. The comparison between original and SiO_2-coated Ag NPs, performed in the same experiment, suggested a coating-independent mitigation effect of bioreactivity exerted by the spray drying procedure. However, once corrected for the actual Ag content of the spray-dried powder, no significant difference was found in the IC_{50} values, indicating that neither silica coating nor spray drying mitigate cytotoxicity. The effects on viability of original TiO_2 and ZrO_2 NPs were assessed using P25 Aeroxide TiO_2 NPs as a reference material. These materials did not affect significantly cell viability at any time point tested, so that it was not possible to estimate IC_{50} values for either cell line. However, titania produced a clear-cut induction of *Nos2* expression in Raw264.7 cells, thus indicating their potential pro-inflammatory activity. Citrate coating did not produce any significant attenuation of the biological effect. In summary, these preliminary results showed no mitigating effect of the surface modifications tested on the biological effects of the engineered NPs investigated. However, the exploitation of this *in vitro* experimental strategy can be useful for the preliminary assessment of the mitigation potential of surface modifications of both low-toxic and high-toxic engineered NPs.

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