Cytotoxicity and biointeractions of nanosilver in macrophages

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ABSTRACT

Nanosilver is a one of the most common engineered nanomaterials in textiles, food packaging, paints and air or water filters demanding a better understanding of its toxicity. Here, the viability of murine macrophages (RAW 264.7) was investigated in the presence of nanosilver using a MTS assay. The nanosilver was immobilized on nanostructured silica to prevent its flocculation during cytotoxicity evaluation. Cell cultures were exposed to 0 – 490 mg/L of pure silica and Ag/SiO_2 nanoparticles containing 50 wt% Ag. The average Ag nanoparticle diameter was closely controlled from 5.7 to 16.8 nm at an Ag mass concentration of 5, 10 and 20 mg/L over 26 h. Nanostructured silica was non-toxic at concentrations below 50 mg/L. Cell viability depended on both Ag size and concentration. The cytotoxicity was dominated by the release of silver ions from the surface of the smaller nanosilver particles. For larger ones, the released ions and the direct contact of cells with the nanosilver surface played a comparable role. Size dependent differences in the cytotoxicity of nanosilver were further confirmed by dark field imaging. Nanosilver was less toxic to murine macrophages than to E. coli bacteria at similar concentrations and particle sizes.

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Keywords: silver, silica, nanoparticles, cytotoxicity, ions

1 INTRODUCTION

Nanosilver is currently a subject of much debate while it is being used in textiles, air conditioners, air purifiers, washing machines and even paints and pigments. It is biologically relevant as both an antimicrobial agent as well as for its plasmonic properties for medical use in diagnostics and therapeutics [1]. The broad use of this nanomaterial bears serious concerns with respect to human health and environment, demanding better elucidation of its properties and toxicity [2-4].

Several studies have demonstrated a reduction in cell viability when cultured cells were exposed to silver nanoparticles in suspension. Braydich-Stolle et al [5] showed strong reduction in the viability of spermatogonial stem cells as assessed by an MTS assay when exposed to suspension of Ag nanoparticles (d = 15 nm). Carlson et al [6] had examined hydrocarbon-coated Ag nanoparticles (d = 15, 30 and 55 nm) in the presence of alveolar macrophages. The smaller sized particles contributed to lower cell viability compared to the larger nanosilver, though the overall toxicity was still less than that of uncoated Ag particles. Foldbjerg et al [7] examined Poly Vinyl Pyrrolidone (PVP) coated silver nanoparticles (d = 68 nm) against THP-1 monocytes and found stronger cytotoxicity. Similar observations were made also by Park [8] et al who investigated the cytotoxicity of silver nanoparticles (d = 68.9 nm) in the presence of murine macrophages. Based on the above, it can be concluded that silver nanoparticles can have a negative impact on cell viability; though overall the values differ substantially, and strongly depend on the preparation and size of the particles. Further the above studies focus on the toxicity exerted by the silver nanoparticles as a whole, whereas recent literature distinguishes between toxicity by the released Ag^+ ions from the surface [9, 10] and contact with the Ag surface itself.

To quantitatively address the latter on mammalian cells such as murine macrophages, a close control of nanosilver size is necessary. Previously, we have shown our capability to closely control the size of silver nanoparticles by stabilizing the nanosilver on silica support [10, 11]. By doing so flocculation in aqueous suspension was prevented, facilitating investigation of Ag^+ ion release in aqueous solutions as well as the antibacterial properties of nanosilver. Ion release was found to be dependent on Ag particle diameter. The antibacterial effects of the release Ag^+ ions in both the presence and absence of silver nanoparticles was examined. The antibacterial effects of smaller nanosilver were dominated by the released ions, whereas the activity of larger nanoparticles was mediated by both the released ions as well as the surface contact [10]. When antibacterial activity was shown as a function of either Ag mass, particle count or specific surface area concentration, highest correlation was observed with the latter [11].

Here the effect of nanosilver size on the viability of mammalian macrophages is investigated, as they are of the first cell lines that nanosilver will encounter in vivo. The toxicity of the silica support is investigated also identifying conditions that render it neutral or inert for the present study. A size and concentration dependent toxicity is observed for nanosilica in mammalian cell lines. Such nanosilver-silica particles are suspended in buffer solutions and the nanosilver toxicity is quantitatively investigated. A focus of this study is on apportioning the nanosilver toxicity contribution to its ions or to the direct contact of cells with the nanosilver surface to better understand how nanosilver-containing products may impact mammalian cells.

2 EXPERIMENTAL

2.1 Particle synthesis

Composite, Janus-like Ag/SiO_2 containing 50% wt Ag and pure SiO_2 particles were generated by flame spray pyrolysis and collected on glass fibers filters previously described in detail [10]. Particles were prepared as such without any surface functionalization or conditioning and were dispersed in deionized water by ultrasonication (Sonics vibra-cell) for one minute at 40% amplitude, with a pulse configuration of on/off 0.5s/0.5s. Sonication was performed twice to ensure all particles were homogeneously dispersed. These particle suspensions or dispersions were thereafter autoclaved at 121°C.

The Ag ion release or leaching from Ag/SiO_2 nanoparticle suspensions (200 mg/L of Ag in deionized water) was measured by an ion selective electrode (801 Stirrer, Metrohm). Solutions containing, however, only released or leached Ag^+ ions were obtained by centrifuging 1.5 ml of the above Ag/SiO_2 nanoparticle suspensions at 15'000 g in a tube for 20 minutes at room temperature. Upon completion of centrifugation, and carefully, to not agitate the nanoparticles' pellet adhering at the bottom of the tube, an 800 µl aliquot was taken from the supernatant, containing the released ions. Samples containing either Ag/SiO_2 particles and Ag^+ ions or only the Ag^+ ions were diluted 1:4 in Hank's Buffered Salt Solution (HBSS) to obtain the desired solutions with total Ag concentration of 5, 10 or 20 mg/L Ag, before applying to the cells.

Murine macrophages (RAW 264.7) were grown in Dulbecco's Modified Eagle Medium (DMEM, 31966-021, Invitrogen), supplemented with 10% Fetal Bovine Serum (FBS, 10270-106, Invitrogen) and 1% Penicillin-Streptomycin solution (15140-122, Invitrogen), which is further referred to as just DMEM. Subcultivation was performed three times a week at a 1:3 ratio. Cells were grown at 37°C in a humidified atmosphere containing 5% CO_2.

The in vitro cell viability was determined by the CellTiter 96 Aqueous One Cell Proliferation MTS Assay (Promega, Madison WI). The macrophages were exchanged into DMEM (31053-028, Invitrogen) supplemented with 10% FBS, 1% Penicillin-Streptomycin solution, 1% Sodium Pyruvate (11360-070, Invitrogen), 2% Glutamax (35050-061, Invitrogen) and further referred to as DMEM without phenol red. The macrophages were seeded at a concentration of 50'000 cells/well and cultured for 24 h. Media was aspired and replaced by 100 µl of HBSS containing a) Ag/SiO_2 nanoparticles of different Ag particle diameters and concentrations (5, 10 and 20 mg/L) with their corresponding Ag^+ ions, b) only Ag ions and c) different SiO_2 nanoparticle concentrations (0 – 490 mg/L). All cells were incubated over a period of 26 hours with the silica nanoparticles dispersed in HBSS buffer. Cells were exposed to Ag/SiO_2 nanoparticle solution for 24 h before adding 20 µl of MTS for 2h. The absorbance of the cell suspensions was measured at 490 nm using a standard plate reader (Tecan Infinite M200) and the cell viability was determined by:

$$\text{Cell viability (\%)} = \frac{A - A_{BG}}{A_C - A_{CBG}} * 100$$

where A represents the absorbance of the wells containing cells treated with nanoparticles and MTS, A_{BG} stands for the background absorbance representing wells without cells treated with the corresponding nanoparticles and MTS. Similarily A_C and A_{CBG} represent the absorbance of wells with and without cells, respectively, treated with buffer solution and MTS only. The tetrazolium compound is bioreduced to a formazan product by dehydrogenase enzymes in metabolically active cells, whose absorbance is directly proportional to the number of living cells in culture.

3 RESULTS & DISCUSSION

3.1 Particle morphology

Figure 1 shows transmission electron microscopy (TEM) images of Ag/SiO_2 nanoparticles, with an Ag particle diameter of (a) 5.7 nm and (b) 16.8 nm. Both images show dark nanosilver particles dispersed on gray amorphous SiO_2 support.It can be seen that the nanosilver particles are much larger in Figure 1b, indicating the god control over the nanosilver size that can be achieved by flame spray pyrolysis [10].

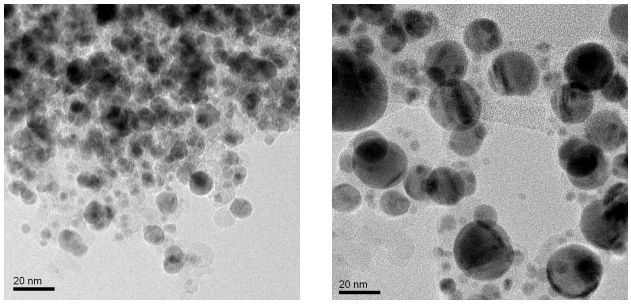

Figure 1. TEM images of nanosilver supported on silica with two different sizes: Average size of 5.7 (a) and 16.8 nm (b).

Silver nanoparticles with closely controlled average size were made on nanostructured silica particles by controlling the Ag concentration in the precursor solution fed to the flame spray unit during their synthesis [10]. The color of the aqueous suspension ranged from light to dark yellow when particle size was increased. Figure 2 shows the fraction of Ag as Ag^+ ions in these suspensions of different average Ag crystalline size, at 200 mg/L total Ag concentration. In agreement with previous studies [10, 11], the Ag^+ ion release is strongly related to the of the Ag nanoparticle size. This can be explained by the higher specific surface area of smaller particles, and thus their easier accessibility to be oxidized, that contributes silver to leaching [10, 11].

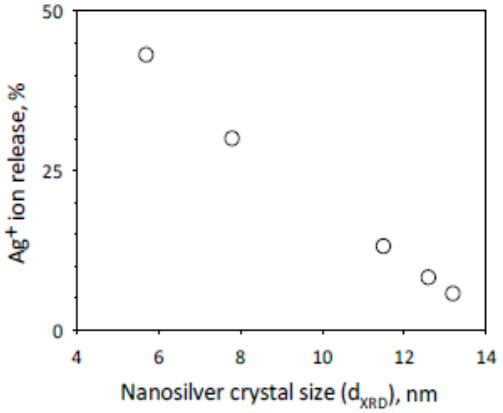

Figure 2. The Ag^+ ion release (%) as a function of different nanosilver average crystal size.

3.2 Silica cytotoxicity

Figure 3 shows how increasing silica concentrations reduce the viability of macrophages. The pure SiO_2 nanoparticles employed here had 300 m^2/g or 9 nm average particle diameter as determined by N_2 adsorption. Up to 50 mg/L, the employed nanostructured silica is practically inert at the employed conditions. Cell viability is reduced progressively, however, when SiO_2 concentrations of above 75 mg/L are applied. Similarly, Brunner et al [12] had noted no toxic effect after 3 days at a concentration of 30 mg/L of slightly larger silica (d_{BET} = 14 nm) in the presence of human mesothelioma and rodent fibroblast cell lines, as assessed by both MTT and DNA tests. Wottrich et al [13] tested silica nanoparticles with an average diameter of 60 nm and 100 nm in human epithelial (A549) and macrophage (THP-1, Mono-Mac6) cell lines using LDH, and observed a concentration dependent toxicity in the A540 and THP-1 cell lines, with the smaller sized silica (d = 60 nm), displaying a maximum relative lethality of roughly 20% at 200 mg/L. By performing LDH and MTT assays in endothelial cell lines, Napierska et al [14] found a 50% reduction in cell viability for the smallest silica particles (14, 15 and 16 nm particle diameter) at concentrations ranging from 33 to 47 mg/L, compared to 89 and 254 mg/L for larger nanosilica (19 and 60 nm).
All these findings are in agreement with the present results. So it was concluded that Ag/SiO_2 nanoparticles could be employed to examine Ag cytotoxicity when 50 mg/L of silica or less was added to the cell solutions at the employed conditions. These conclusions set the focus of this study to comparing the cytotoxic effects of differently sized Ag nanoparticles immobilized on nanostructured silica at Ag mass concentrations ranging from 5 to 20 mg/L, on the present mammalian cell cultures.

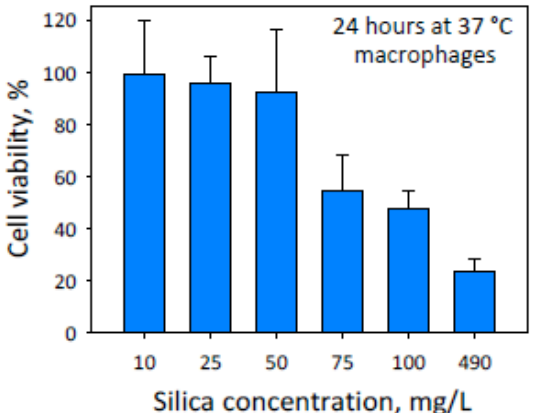

Figure 3. Murine macrophages cell viability by MTS assay in the presence of different concentrations of silica nanoparticles.

3.3 Nanosilver cytotoxicity

Figure 4 shows the cell viability at 10 mg/L initial Ag concentration (prior to centrifugation). At small average Ag particle sizes (less than 10 nm), there is no or little statistical difference in cell viability in the presence of ions regardless of the presence or absence of Ag nanoparticles. Given that the Ag ions constitute of 43% and 30% of the Ag mass concentration (Figure 3) in the suspensions containing Ag nanoparticles of 5.7 and 7.8 nm, respectively, average particle diameter, clearly at these sizes the Ag fraction in the form of ions is far more toxic than contact with the in the Ag particles themselves. This picture changes, however, with suspensions of larger Ag nanoparticles. For example, when the average Ag particle size is 12.6 nm, the cell viability in the absence of Ag nanoparticles is nearly 100% while in their presence drops to 55%. Clearly the contact of cells with the surface of Ag nanoparticles is most detrimental to the cell viability for these Ag particle and ion suspensions.

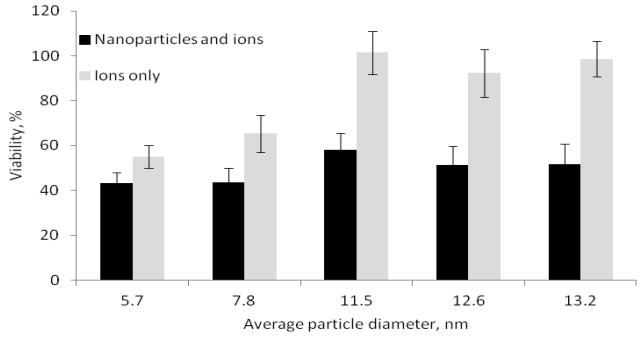

Figure 4: Cell viability of RAW 264.7 macrophages exposed for 26 h to 10 mg/L Ag content of Ag/SiO_2 nanoparticles before (Ag^+ ions and particles, filled bars) and after centrifugation (Ag^+ ions only, open bars).

4 CONCLUSIONS

Silver nanoparticles dispersed within amorphous silica have been synthesized using FSP. Pure silica was found to show reduced viability at concentrations above 50 mg/L, so all experiments were performed at lower silica mass concentrations.

With silver nanoparticles of closely controlled size, we found smaller nanoparticles (d_{XRD} < ~10 nm) showed a small difference in the cell viability in the presence and absence of Ag particles, indicating that Ag^+ ions dominate the cell toxicity. For increasing size (d_{XRD} > ~10 nm), the particles play an increasing role for cell cytoxicity. This is in alignment with data from antibacterial studies.

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REFERENCES

[1] G. A. Sotiriou, S. E. Pratsinis, *Curr. Opin. Chem. Eng.* **2011**, *1*, 3.

[2] G. A. Sotiriou, E. Diaz, M. S. Long, J. Godleski, J. Brain, S. E. Pratsinis, P. Demokritou, *Nanotoxicology* **2011**, *in press*.

[3] G. A. Sotiriou, A. M. Hirt, P. Y. Lozach, A. Teleki, F. Krumeich, S. E. Pratsinis, *Chem. Mater.* **2011**, *23*, 1985.

[4] G. A. Sotiriou, T. Sannomiya, A. Teleki, F. Krumeich, J. Vörös, S. E. Pratsinis, *Adv. Funct. Mater.* **2010**, *20*, 4250.

[5] L. Braydich-Stolle, S. Hussain, J. J. Schlager, M.-C. Hofmann, *Toxicological Sciences* **2005**, *88*, 412.

[6] C. Carlson, S. M. Hussain, A. M. Schrand, L. K. Braydich-Stolle, K. L. Hess, R. L. Jones, J. J. Schlager, *J. Phys. Chem. B* **2008**, *112*, 13608.

[7] R. Foldbjerg, P. Olesen, M. Hougaard, D. A. Dang, H. J. Hoffmann, H. Autrup, *Toxicol. Lett.* **2009**, *190*, 156.

[8] E.-J. Park, J. Yi, Y. Kim, K. Choi, K. Park, *Toxicol. Vitro* **2010**, *24*, 872.

[9] E. Navarro, F. Piccapietra, B. Wagner, F. Marconi, R. Kaegi, N. Odzak, L. Sigg, R. Behra, *Environ. Sci. Technol.* **2008**, *42*, 8959.

[10] G. A. Sotiriou, S. E. Pratsinis, *Environ. Sci. Technol.* **2010**, *44*, 5649.

[11] G. A. Sotiriou, A. Teleki, A. Camenzind, F. Krumeich, A. Meyer, S. Panke, S. E. Pratsinis, *Chem. Eng. J.* **2011**, *170*, 547.

[12] T. J. Brunner, P. Wick, P. Manser, P. Spohn, R. N. Grass, L. K. Limbach, A. Bruinink, W. J. Stark, *Environ. Sci. Technol.* **2006**, *40*, 4374.

[13] R. Wottrich, S. Diabaté, H. F. Krug, *International Journal of Hygiene and Environmental Health* **2004**, *207*, 353.

[14] D. Napierska, L. C. J. Thomassen, V. Rabolli, D. Lison, L. Gonzalez, M. Kirsch-Volders, J. A. Martens, P. H. Hoet, *Small* **2009**, *5*, 846.