Assessment of silver nanoparticle (AgNP) suitability for use in polystyrene-based medical devices

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Introduction

Silver nanoparticles (AgNP) are one of the most commercialized types of nanomaterials, with a wide range of applications owing to their antimicrobial activity. They are particularly important in hospitals and other healthcare settings, where they are used to maintain sterility of surfaces, textiles, catheters, medical implants, and more. While the potential toxicity of AgNP is an understood risk, there is a lack of data on their toxicity in combination with polymeric materials, especially plastic nanoparticles, such as polystyrene nanoparticles (PSNP). In this study, toxicity of PVP-coated AgNP combined with PSNP was tested on Jurkat cells. Cytotoxicity, induction of apoptosis generation of oxidative stress, uptake efficiency and intracellular localization were selected as exposure biomarkers. All experiments were performed with and without serum proteins in the cell culture medium.

Materials & Methods

Nanoparticle synthesis and characterization

Commercial PSNP were used in the study, while AgNP were synthesized by reducing $AgNO_3$ with $NaBH_4$ using poly(vinyl)pyrrolidone (PVP) for surface coating. Size, surface charge and shape of NP was determined using dynamic (DLS) and electrophoretic light scattering (ELS), transmission electron microscopy (TEM) and atomic force microscopy (AFM).

Confocal microscopy

Cells treated with 1 mg/L AgNP and 10 mg/L of fluorescent PSNP for 1 h were stained with phalloidin–TRITC and Hoechst 33342. Samples were acquired on Leica TCS SP8 X microscope.

Atomic Force Microscopy

Force spectroscopy experiments were performed using Nanowizard IV system. Young modulus (*E*) was calculated by fitting the force-indentation curves to the Hertz model.

Oxidative stress assays

ROS generation after 1 h treatment was quantified using 2′,7′-dichlorofluorescein diacetate (DCFH-DA). Monochlorobimane (MCB) assay was used to determine total cellular glutathione (GSH), and rhodamine 123 (Rh123) assay to analyze mitochondrial membrane integrity.

Flow cytometry

Cells were incubated with nanoparticles for 24 h, after which samples were prepared for flow cytometry by collecting cells and staining them with Annexin V Kit.

Table 1. Hydrodynamic diameter (d_H, in nm) and z potential values (mV) for individual particle types and 1:1 mixture of AgNP (at 1 mg Ag/L) and PSNP (at 1 mg/L), obtained after 24 h incubation of particles in different media.

Particle type	Parameter	Media		
		Ultra-pure water	RPMI1640	RPMI/10% FBS
AgNP	d_H (nm)		881.5 ± 130.4	119.2 ± 4.0
	(% population)	72.1 ± 7.3 (100%)	(100%)	(82%)
				21.0 ± 2.4 (18%)
	z potential (mV)	-35.9 ± 4.5	-9.3 ± 0.3	-9.7 ± 0.2
	% released Ag	5.13 ± 2.93	Not detected	Not detected
PSNP	d_H (nm)		46.5 ± 29.5 (45%)	18.5 ± 3.5
	(% population)	19.4 ± 0.5 (100%)	162.4 ± 51.8 (21%)	(100%)
			674.3 ± 183.4 (34%)	
	z potential (mV)	-39.7 ± 10.8	-22.9 ± 2.3	-9.3 ± 0.9
PSNP/AgNP mixture	d_H (nm)	65.7 ± 7.8 (88%)	912.7 ± 86.6 (80%)	119.5 ± 1.4
	(% population)	18.9 ± 4.0 (12%)	139.7 ± 0.0 (20%)	(100%)
	z potential (mV)	-29.0 ± 0.5	-13.1 ± 0.7	-9.2 ± 0.5
	% released Ag	5.0 ± 2.2	Not detected	Not detected

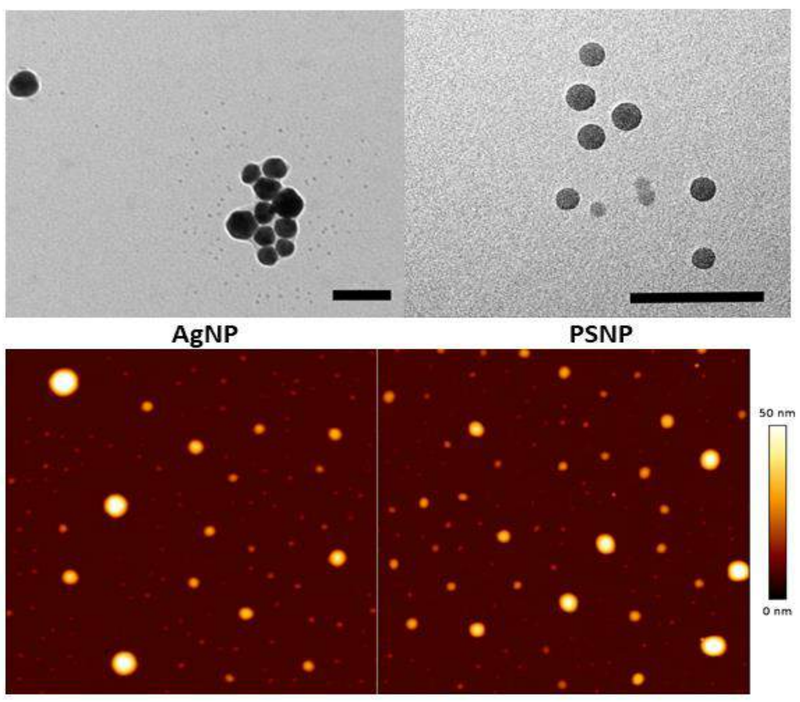

Figure 1. Upper row - Transmission electron microscopy (TEM) images of AgNP (left) and PSNP (right) dispersed in ultrapure water. Scale bars are 100 nm. Bottom row – Atomic force microscopy (AFM) images of individual AgNP (left) and PSNP (right). Scan size is 4 μm for both images. Provided height scale applies to both images.

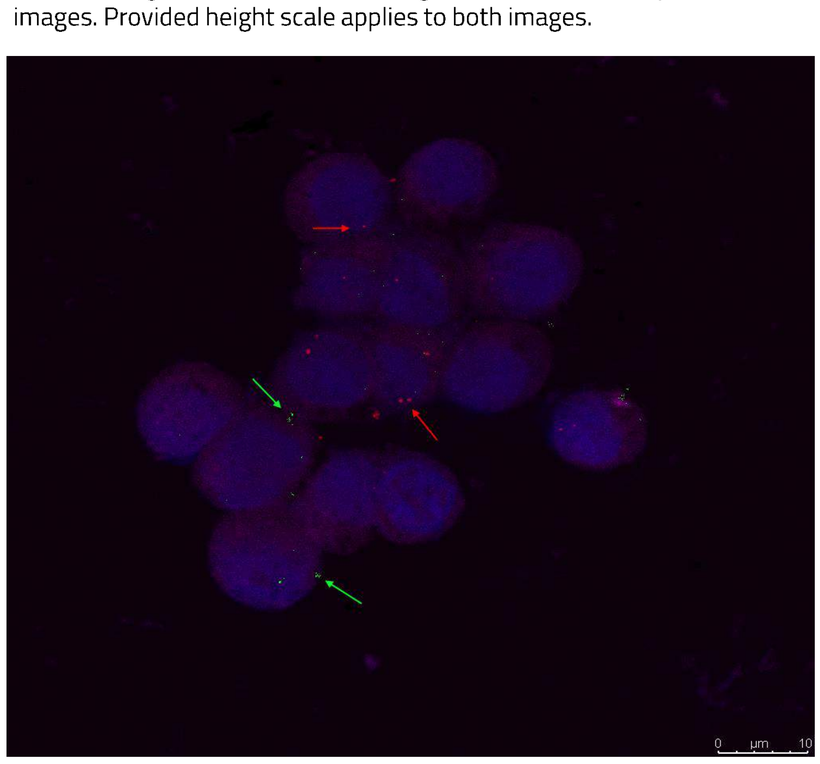

Figure 5. Confocal image of Jurkat cells treated with AgNP/PSNP mixture for 1 h. Cell nuclei are shown in blue, actin is shown in magenta, PSNP are shown in green, and AgNP are shown in red.

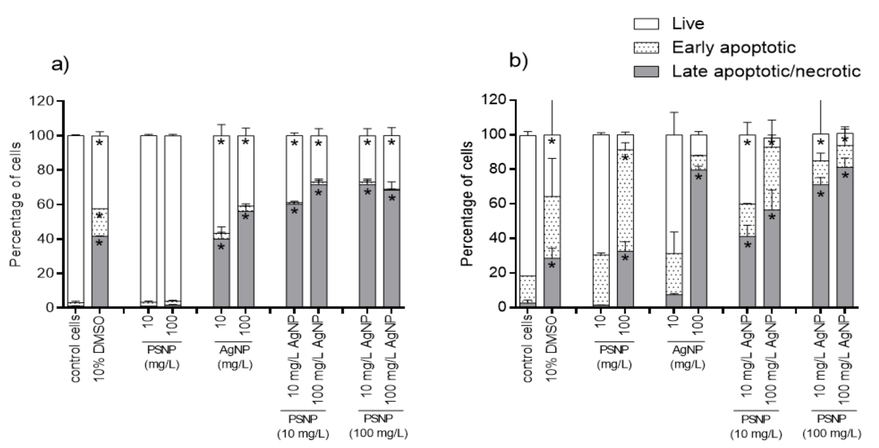

Figure 2. Flow cytometry results after cell treatment with different NP doses for 24 h in cell culture medium a) supplemented with 10% FBS and b) without FBS. Significant results are denoted with an asterisk (*) (P<0.05).

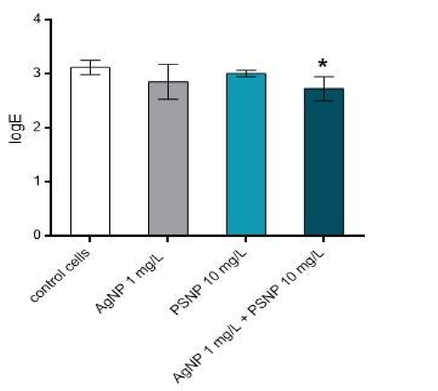

Figure 3. Logarithms of Young's modulus values (logE) for NP-treated cells. Significant results are denoted with an asterisk (*) (P<0.05).

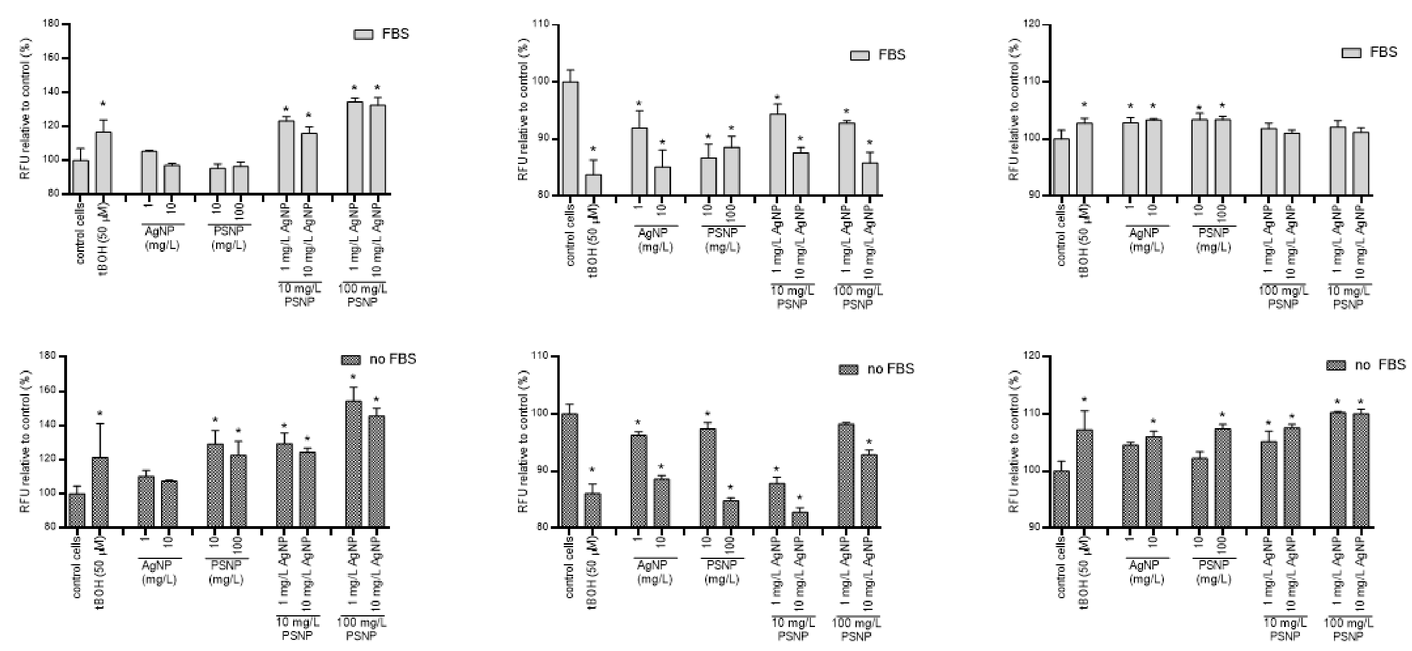

Figure 4. Results of oxidative stress response of NP-treated cells: A – DCF assay results; B – MCB assay results; C – Rhodamine 123 assay results. Significant results are denoted with asterisk (*).

Results

Data presented here shows detrimental effects of AgNP and PSNP on Jurkat cell viability, increase in ROS production and subsequent apoptosis induction. Although both nanoparticle types share the same mechanism of toxicity, i.e. ROS generation and subsequent cellular damage, a decrease in cell viability exceeded the expected additive effect of total nanoparticle concentration. Jurkat cells treated with AgNP/PSNP mixture at concentrations that were not toxic when these NP were applied alone showed increased ROS production, apoptosis and death cells. Combined effect of AgNP and PSNP treatment also significantly modulated mechanical properties of Jurkat cells by reducing cell stiffness. Results presented here show that toxicological profiles of NP mixtures can differ significantly from profiles of individual NP types.

Acknowledgments

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