Role of nanoparticle surface charge in their toxicity

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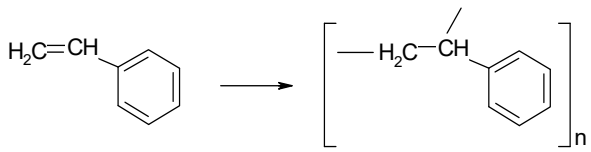
Abstract. Dust often contains chemical airborne pollutants that might negatively affect human health. Polystyrene is one of the most widely used types of plastic. Bulk polystyrene exhibits no short-term cytotoxicity. However, during degradation of polystyrene, small nanoparticles are formed. Due to specific properties, such as the large surface to volume ratio, toxicity of nanoparticles might be different to that of the bulk material. Hence, particularly the surface chemistry is crucial for nanoparticle behaviour in biological environment. For this study, carboxyl- (PS-COOH) and amino-functionalized (PS-NH_2) nanoparticles were prepared by free-radical copolymerization in a direct (oil in water) miniemulsion system. We show that surface functionalization of polystyrene nanoparticles with amino groups (PS-NH_2), but not with carboxyl groups (PS-COOH), induced inhibition of proliferation in a monocytic cell line and induce a specific cell death, apoptosis. In PS-NH_2–treated cells, acidic vesicular organelles exhibited elevated pH and impaired processing of a lysosomal enzyme. Moreover, solely in PS-NH_2-treated cells, but not in PS-COOH-treated cells, this was followed by permeabilization of acidic vesicular organelles and induction of cell death. These data indicate that surface charge of nanoplastics defines their effects on biological systems and can be used to predict environmental toxicity of nanoplastics.

1 Introduction

Nanoparticles are defined as particles of any shape limited in size to 100 nm in either two or three dimensions [1]. An outcome of the small size is that the percentage of atoms located on the surface of a nanoparticle become significant compared to the ones of the bulk. The result is a material that is mainly characterized by the properties of its surface atoms and electrons including features like quantum effects. Polystyrene is one of the most widely used types of plastic. Bulk polystyrene is among the least toxic, when compared to other natural and synthetic building materials [2].

Changes in size or the surface of the particles result in drastic changes in the behavior of nanoparticles,



Styrene Polystyrene

particularly in a biological environment [3].

Macrophages are key players of immunity and are a first line of defense against pathogens on different portals of entry into the body. In our studies we compared the effects of the chemical surface decoration of polystyrene nanoparticles on human macrophages.

2 Methods

The nanoparticles were synthesized by mini emulsion polymerization and were of approximately the same hydrodynamic diameter of about 120 nm. They were functionalized either with roughly the same amount of carboxyl (PS-COOH) or amino (PS-NH_2) groups. The particles were characterized for average particle size, polydispersity index (PDI), and ζ-potential by using a Zetasizer Nano (Malvern Instruments); the measurements were performed in PBS, pH 7.4. The surface charge density was measured by polyelectrolyte titration [2].

The human monocytic cell line THP-1 from American Type Culture Collection was cultured as recommended and differentiated into macrophages with PMA [4].

Cellular viability was analyzed by reduction of XTT by mitochondrial dehydrogenases (Roche Diagnostics, Basel, Switzerland). Phosphatidylserine expression as an early sign of apoptosis was determined by flow cytometric analysis of binding of fluorescein isothiocyanate-labeled annexin V (Roche); propidium iodide was used to differentiate necrotic cells.

The integrity of acidic vesicular organelles in cells treated with nanoparticles was assessed by acridine orange staining and analysis of red fluorescence of the aggregated form of the dye by using flow cytometry [4]. The inhibitor of v-ATPase bafilomycin A1 was used to explore the role of v-ATPase. Apoptosis analysis in

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THP-1 cells treated with nanoparticles and double-stained with annexin V-FITC/propidium iodide (Roche) was performed by using flow cytometry (FACSVerse, BD Biosciences).

3 Results

The polystyrene nanoparticles used in this study were functionalized either with carboxyl (PS-COOH) or amino (PS-NH_2) groups. The physicochemical characterization of the particles is summarized in Table below [2] Table 1).

Table 1. Physicochemical characteristics of functionalized polystyrene nanoparticles.

Particle	Diameter nm	PDI, Polydisper-sity index	Groups per particle (per nm^2)	Zeta-potential mV
PS-COOH	119 ± 19	0.002	6406 (0.144)	- 36.2
PS-NH_2	117 ± 17	0.008	5630 (0.130)	54.4

Both kinds of particles had a similar mean hydrodynamic diameter of about 120 nm and remained monodisperse in aqueous conditions as reflected by the low polydispersity index. Functionalization with either carboxyl or amino groups resulted in either highly negative or positive ζ-potentials.

PS-COOH particles exhibited no toxic effect on cells (Fig. 1). Analysis demonstrated that PS-COOH did not affect the THP-1 cell proliferation, whereas PS-NH_2 particles virtually immediately terminated the cell division [5]. PS-NH_2 particles not only inhibited proliferation of THP-1 cells but after 3 days induced exposure of phosphatidyl serine on the outer membrane leaflet of THP-1 cells consistent with the induction of apoptosis. It is also notable, that the cell size decreased after treatment with positively charged PS-NH_2 particles similarly of that of cells treated with camptothecin, an inhibitor of topoisomerase I, used as a positive control (Fig. 1).

Release or lysosomal enzymes may damage mitochondria and elicit increased ROS formation under such conditions. Damaged mitochondria and high levels of ROS can trigger apoptosis [7]. Indeed, further we showed that lysosomal damage and activation of vacuolar ATPase is central to PS-NH_2 induced toxicity.

Thus, bafilomycin A1, an inhibitor of vacuolar ATPase known to prevent acidification of lysosomal compartments, concentration-dependently inhibited the lysosomal rupture and apoptosis induced by exposure of THP-1 monocytes and differentiated THP-1 cells (macrophages) to PS-NH_2 (Fig. 2) indicating the causative role of the lysosomal dysfunction in the apoptosis induced by nanoparticles functionalized with amino groups.

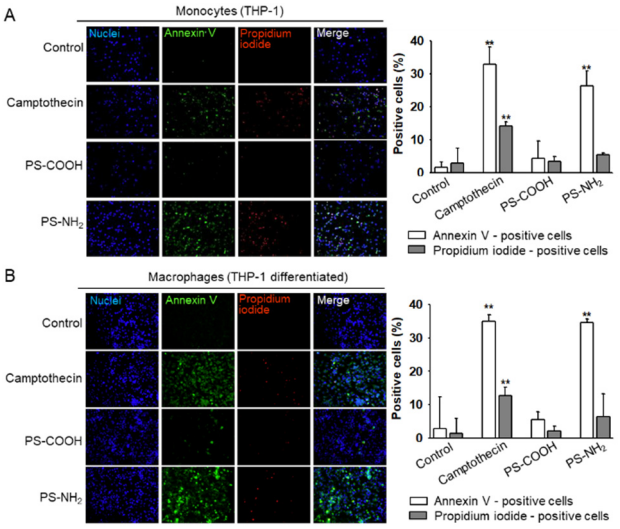

Fig. 1. Amino-functionalized polystyrene nanoparticles induce apoptotic cell death. THP-1 (A) and differentiated THP-1 (B) were stimulated with either PS-COOH or PS-NH2 (each at 100 µg/ml) for 72 h, analyzed by fluorescence microscopy and quantified using ImageJ. The graphs show amount of apoptotic (annexin V+) and late apoptotic or necrotic (propidium iodide+) cells. Campthotecin – positive control. Mean ± SEM, n = 3, **p < 0.01 (http://creativecommons.org/licenses/by/2.0 [2]).

This study revealed another interesting aspect concerning the activation of the mammalian target of rapamycin (mTOR), a key kinase controlling cell growth and proliferation and implicated in many human diseases including cancer and diabetes [6]. Thus, the integrity of membranes of acidic lysosomal compartments are important for the activation of mTOR [6]. We could show that PS-NH_2 inhibit, whereas PS-COOH activate the mTOR signaling in leukemia cells. Consistently, PS-NH_2 inhibit activation of the mTOR downstream targets, Akt and p70 ribosomal S6 kinase 1, and blocked proliferation in three leukemia cell lines *in vitro* and *in vivo* [5].

4 Conclusion

These studies show that, although, polystyrene has been claimed to be nontoxic, surface-charged nanosized polystyrene particles may behave totally different from the bulk material. The surface chemistry plays a crucial role determining the impact of nanoparticles on diverse biological systems. The amino-functionalized particles can be regarded as a model for cationic nanoparticles, and the carboxyl-functionalized, as a model for anionic particles to predict the environmental toxicity of microplastics. The toxicity of cationic nanoparticles might be controlled by reduction of the amount of positive charged groups on the particle surface

By careful consideration of potential side effects, it will be possible to develop new materials overcoming the problems of conventional plastics and also to evade possible risks for the environment.

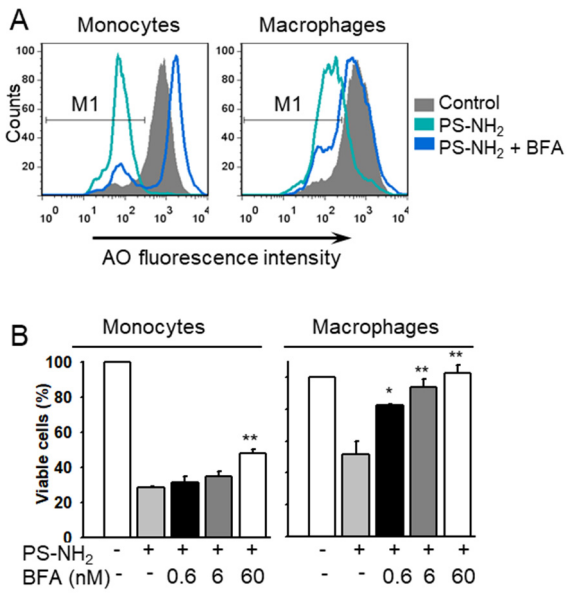

Fig. 2. Inhibition of vacuolar ATPase by bafilomycin A1 antagonizes the toxic effect of PS-NH2 nanoparticles. (A) Analysis of lysosomal permeabilization of cells stimulated with PS-NH2 (100 µg/ml) with or without bafilomycin A1 (BFA, 6 nM) for 72 h. After treatment with nanoparticles, cells were stained with acridine orange (AO) and analyzed by flow cytometry. M1 gating was used to assess the number of AOlow cells with leaky lysosomes. (B) Analysis of cell viability. Cells were treated as in A and analyzed by XTT assay. Results are mean ± SEM, n = 3, *p < 0.05, **p < 0.01. (http://creativecommons.org/licenses/by/2.0 from [2]).

Abbreviations

FITC	fluorescein isothiocyanate
mTOR	mammalian target of rapamycin
PBS	phosphate buffered saline
PDI	polydispersity index
PMA	phorbol-12-myristate-13-acetate
PS-COOH	carboxyl-functionalized polystyrene nanoparticles
PS-NH2	amino-functionalized polystyrene nanoparticles
v-ATPase	vacuolar-type H+ -ATPase
XTT	(2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide)

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