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Role of bovine serum albumin and humic acid in the interaction between SiO_2 nanoparticles and model cell membranes☆

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ABSTRACT

Silica nanoparticles (SiO_2 NPs) can cause health hazard after their release into the environment. Adsorption of natural organic matter and biomolecules on SiO_2 NPs alters their surface properties and cytotoxicity. In this study, SiO_2 NPs were treated by bovine serum albumin (BSA) and humic acid (HA) to study their effects on the integrity and fluidity of model cell membranes. Giant and small unilamellar vesicles (GUVs and SUVs) were prepared as model cell membranes in order to avoid the interference of cellular activities. The microscopic observation revealed that the BSA/HA treated (BSA-/HA-) SiO_2 NPs took more time to disrupt membrane than untreated-SiO_2 NPs, because BSA/HA adsorption covered the surface Si–OH/Si–O^- groups and weakened the interaction between NPs and phospholipids. The deposition of SiO_2 NPs on membrane was monitored by a quartz crystal microbalance with dissipation (QCM-D). Untreated- and HA-SiO_2 NPs quickly disrupted the SUV layer on QCM-D sensor; BSA-SiO_2 NPs attached on the membranes but only caused slow vesicle disruption. Untreated-, BSA- and HA-SiO_2 NPs all caused the gelation of the positively-charged membrane, which was evaluated by the generalized polarity values. HA-SiO_2 NPs caused most serious gelation, and BSA-SiO_2 NPs caused the least. Our results demonstrate that the protein adsorption on SiO_2 NPs decreases the NP-induced membrane damage.

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1. Introduction

Silica nanoparticles (SiO_2 NPs) are increasingly used in inorganic paints, semiconductors, drug delivery and medical imaging due to their special physiochemical properties (Lu et al., 2007, 2010; Trewyn et al., 2008). Their application raises the chance of possible exposure to human beings. Studies have reported that the inhale of SiO_2 NPs can cause health problems, such as granuloma formation, silicosis, emphysema and even lung cancer (Attfield and Costello, 2004; Maynard and Kuempel, 2005; Napierska et al., 2010; Pisani et al., 2015).

The knowledge on the interaction between NPs and cell membrane is important to the toxicity mechanisms and the safe applications of SiO_2 NPs. Cell membrane is the first contacting site when cells are exposed to NPs. Oxide NPs have been reported to change the molecular structure of proteins and lipids (Jiang et al., 2010), thus can influence the cell membrane morphology and function. Intact and fluid plasma membrane is essential to maintain cellular physiological activities. The intact membrane isolates the intracellular environment and keeps the normal cell metabolism. NPs can disrupt intact membrane as suggested by the microscopic observation (Laurencin et al., 2010; Olubummo et al., 2013; Liang et al., 2014) or dye diffusion assay (Leroueil et al., 2007). Membrane fluidity is necessary for substance exchange between intra- and extracellular environment, which can be either increased or reduced after NP exposure (Park et al., 2006; Santhosh et al., 2014).

Human beings or other organisms are rarely exposed to bare NPs, because NPs adsorb the widely existent natural organic matter (NOM) after their release into natural waters or soils, and interact with biomolecules in interstitial fluid after the uptake by organisms. The physiochemical properties of SiO_2 NPs with NOM/biomolecular coatings are different from the bare particles in their size, charge, stability, surface functional groups, etc (Schwabe et al., 2013; Yallapu et al., 2015). The cellular uptake and cytotoxicity of NPs are also changed by the adsorption of NOM/biomolecules (Ge et al., 2011; Ruge et al., 2011; Lin et al., 2012). The adsorbed NOM/biomolecules form a physical barrier between NPs and cell surface, which can prevent the NPs from contacting cell surface or

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producing reactive oxygen species (ROS) (Lin et al., 2012; Yallapu et al., 2015). However, the enhanced NP toxicity after NOM adsorption also has been reported due to the increasing oxidative stress and the release of toxic cations (Wang et al., 2011; Yang et al., 2013). Considering the complicated influences from NOM/biomolecules and the inconsistent toxicity reports, how the NOM/biomolecule-treated SiO_2 NPs interact with cell membrane should be addressed. It is a key step related to both the cellular uptake and cytotoxicity of SiO_2 NPs in the real environment. Up to date, the role of surface coatings on the SiO_2 NP mediated cell membrane damage is largely unknown.

Humic acids (HAs) are widely existent NOM and proteins are the most important group of biomolecules in body fluid. Therefore HA and bovine serum albumin (BSA) are selected to represent the NOM and biomolecules, respectively. This work aims to study the influence of BSA/HA adsorption on the SiO_2 NP-membrane interaction, and on the membrane integrity and fluidity. Phospholipid vesicles are prepared as model membranes to avoid the uncertain influences of cellular physiological activities. This study will provide better understanding on the potential physical mechanism of toxicity.

2. Materials and methods

2.1. Materials

Porous type ($P-SiO_2$) and sphere type silica ($S-SiO_2$) NPs were purchased from Zhejiang Hongsheng Material Technology Co., China. The two types of silica NPs are most widely-used industrial nanomaterials but have different surface properties. The phospholipids 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-Dioleoyl-*sn*-Glycero-3-[Phopho-rac-(1-glycerol)] (Sodium Salt) (DOPG), 1,2-dipalmitoyl-3-trimethylammonium-propane (chloride salt) (16:0 TAP), and fluorescent lipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (RhB-PE) were bought from Avanti Polar Lipids (Alabaster, AL, USA). Fluorescence probe 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan) was purchased from Molecular Probes (Eugene, OR, USA). Bovine serum albumin (BSA) was purchased from Sigma (St. Louis, MO, USA) and HA was provided by Aladdin Reagent (Shanghai, China).

2.2. Silica NP characterization and treatment

The total surface areas (S_{BET}) of $P-SiO_2$ and $S-SiO_2$ NPs were measured using the multi-point Brunauer-Emmett-Teller (BET) method from the N_2-adsorption isotherms at 77 K with a NOVA 2000e instrument (Quantachrome, USA). The internal micro-pore surface areas (S_{pore}) and external surface areas (S_{ext}) were calculated from N_2-sorption isotherms by the t-plot method (Lowell and Shields, 1991). S_{BET}, S_{pore} and S_{ext} of $P-SiO_2$ NPs are 570 m^2/g, 434 m^2/g and 133 m^2/g. S_{BET}, S_{pore} and S_{ext} of $S-SiO_2$ NPs are 191 m^2/g, 65 m^2/g and 123 m^2/g. The content of hydroxyl groups on the two SiO_2 NPs were quantified by the titration method (Dai et al., 2015), and the experimental details of titration were introduced in the supporting information (SI). $P-SiO_2$ and $S-SiO_2$ NPs contain 1.1 ± 0.1 OH/nm^2 and 1.0 ± 0.1 OH/nm^2, respectively.

Silica NPs of 5000 mg/L were shaken in 3000 mg/L BSA or HA for 24 h to prepare the BSA-treated or HA-treated SiO_2 NPs (BSA-SiO_2 or HA-SiO_2 NPs). Unadsorbed BSA or HA was removed by centrifugation at 2400*g* for 30 min. The NPs were washed twice by deionized (DI) water and then were freeze dried. The process to calculate the BSA or HA adsorption amount was introduced in SI. The adsorption amounts of BSA on $P-SiO_2$ and $S-SiO_2$ NPs are 101.8 and 66.8 mg/g, respectively. The adsorption amount of HA on $P-SiO_2$ and $S-SiO_2$ NPs are 17.0 and 14.6 mg/g, respectively.

Zeta potentials (pH 3–9) and the hydrodynamic diameters (d_H) of NPs were measured at 25 °C by a Malvern zeta-sizer (Nano ZS90, Malvern Instruments Ltd., UK). The SiO_2 NP morphologies were imaged under a transmission electron microscopy (TEM, JEM-1011, JEOL, Japan) after drying a small drop of NP suspension on the copper grid. The sedimentation curves of 500 mg/L NPs are measured in 0.1 *M* glucose by the optical absorbance at 800 nm. After sonication, samples of 3 mL were added in a square quartz cuvette. The absorbance intensity (A) was continuously record for 3 h, then measured at 12 h and 24 h. The intensity was modified by the initial intensity (A_0).

2.3. Vesicle preparation

Giant unilamellar vesicles (GUVs) were prepared by the gentle hydration method and small unilamellar vesicles (SUVs) were produced by the extrusion method (Akashi et al., 1996; Cho et al., 2010). Because cell membrane contain both negatively-charged and positively-charged functional groups (Lewinski et al., 2008), negatively-charged DOPG or positively-charged 16:0 TAP were added into membrane to simulate the charged groups in the cell membrane. Therefore, DOPC were mixed with 10% DOPG or 16:0 TAP in chloroform:methanol (2:1, v:v) to prepare $GUVs^-$ or $GUVs^+$. Then the phospholipid mixture was dried in a rotary glass vial under N_2 flow to form a thin film inside the vial, and was kept in vacuum for more than 2 h to remove the solvent residues. To make GUVs, the phospholipid film was incubated in 0.1 *M* sucrose at 40 °C for 24 h (Wei et al., 2015). Fluorescent GUVs were prepared by adding 0.1% (w/w) of RhB-PE into lipid solutions before gentle hydration. To make SUVs, phospholipid film was incubated in DI water for 1-h gentle hydration, and then the solution was pushed through a 100 nm filter in an extruder for more than 25 times. GUVs were used for microscopic observation, while SUVs were used for quartz crystal microbalance with dissipation (QCM-D) monitoring and fluorescence spectra measurement.

2.4. Nanoparticle exposure and GUV morphology observation

Giant unilamellar vesicles were diluted to 0.5 mg/mL in 0.1 *M* glucose, exposed to 50 mg/L NP suspensions at 25 °C in a glass bottom (30 mm diameter, 0.15 mm thickness) Petri dish. The interaction of NPs with membrane was observed by an inverted microscope at bright field. The amount of GUVs after NP exposure was counted according to the method in SI. Aim to study the GUV morphologic changes, fluorescently labeled GUVs were applied and the exposure process was recorded by a Carl Zeiss LSM 700 fluorescence confocal microscope.

2.5. Deposition kinetics of SiO_2 NPs on the SUV layer monitored by QCM-D

The deposition kinetics of untreated, BSA- and HA-SiO_2 NPs on membrane were monitored by a QCM-D (E4, Q-Sense, Sweden). The system was mounted by four sensor flow modules. Au-coated quartz crystal sensors (QSX301, Q-Sense) were housed in modules. Before the deposition experiment, Au sensors were rinsed with a freshly prepared solution of $H_2O:H_2O_2:NH_4OH$ (5:1:1 by volume) and DI water, followed by N_2 flow to remove the droplets and then treated with UV/ozone for 15 min. Meanwhile, SUVs were diluted to 0.1 mg/mL in Tris/NaCl buffer (10 m*M* Tris, 150 m*M* HCl,

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