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Effect of oxide nanoparticles on the morphology and fluidity of phospholipid membranes and the role of hydrogen bonds

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ABSTRACT

Engineered oxide nanoparticles (NPs) are widely applied in insulators, catalyzers, paints, cosmetic products, textiles and semiconductors. Their attachment on cell membrane may lead to cytotoxicity. The effects of Al_2O_3, Fe_2O_3, SiO_2, TiO_2 and ZnO NPs on membrane integrity and fluidity were studied using giant or small unilamellar vesicles in this study. Al_2O_3 and SiO_2 NPs disrupted the oppositely charged membrane, indicating the important role of electrostatic attraction. However, Fe_2O_3, TiO_2 and ZnO NPs did not cause serious membrane disruption as Al_2O_3 and SiO_2 NPs. Membrane fluidity was evaluated by the generalized polarity (GP) values of Laurdan fluorescent emission. SiO_2 NPs induce the membrane gelation of both positively and negatively charged membrane. Al_2O_3 and ZnO NPs induced the gelation of the oppositely charged membrane, but did not cause obvious membrane gelation to the like charged membrane. The phospholipid molecular structural changes after NP exposure were analyzed by Fourier transform infrared (FT-IR) spectroscopy. FT-IR spectra revealed the hydrogen bond formation between NPs and the carbonyl/phosphate groups of phospholipids. Al_2O_3 and SiO_2 NPs showed strongest evidence of hydrogen bonding on their FT-IR spectra. It was consistent with the microscopic observation and fluorescent data that Al_2O_3 and SiO_2 NPs caused more serious membrane disruption and gelation. This study on membrane damage provides further knowledge on the cytotoxicity of nanomaterials and the safety of NP application.

Introduction

The growing interest for nano–bio interface interactions is motivated by the development of nanotechnology, biotechnology and medicine, also is due to the concern to the health risk associated with nanomaterial application. Nanoparticle (NP)-induced toxic effects have already been identified and drawn much attention (Krishnaraj et al., 2016; Henderson et al., 2016), but further investigation is required to examine fundamental mechanisms underlying these undesirable biological responses. Among the predictive pathways leading to NP toxicity, the damage to cell membrane is one of the main concerns.

The contact of the NPs with the cell membrane carries the risk of membrane disruption and cytotoxic effects. Indeed, the lactate dehydrogenase (LDH) release from cells has been observed for a wide range of NPs (Uo et al., 2005; Li et al., 2015), indicating the plasma membrane damage. Perturbation of the membrane potential has been revealed when positively charged Au NPs bind to the plasma membrane, and the membrane Ca^{2+} influx has been elevated (Arvizo et al., 2010). Hemolysis can be caused by silica NPs due to the membrane damage of red blood cells (Thomassen et al., 2011). Despite many cytotoxicity reports, it remains uncertain how the cell membrane is damaged by NPs. Even the relationship is

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unclear between cell membrane leakage and the NP exposure. The cell membrane leakage is possibly caused by the direct NP attachment, also possibly caused by the changes in cellular physiological activities. Moreover, the biomolecules in the cell incubation media may change the NP surface properties, which increases the complexity and the uncertain factors in the system. In addition, the variation in cell lines also makes differences in the NP-membrane interactions, which may cause problems in the comparison between nanotoxicology studies (Rothen-Rutishauser et al., 2006). Therefore the model phospholipid membranes are needed to explore the mechanisms of NP-membrane interaction and to avoid the variation in cell lines or in incubation media. Giant unilamellar vesicles (GUVs) appear as cell-like shape and size (1 to 100 μm), are large enough to be visible using optical microscopes. Therefore they are often used to mimic the cell membranes in a simplified environment (Laurencin et al., 2010; Jiang et al., 2017). GUVs offer the possibility to test the various lipid mixtures, and allow us to tune the global net charge of the membrane, which favors the study on the mechanisms of the NP-membrane interactions.

Recently, interactions between NPs and phospholipid bilayers have been investigated in some researches using model membranes. The attachment of NPs can increase the permeability of phospholipid bilayers (De Planque et al., 2011), and cause the membrane rupture (Zupanc et al., 2012). The fluidity of the membrane has been reported to change due to NP exposure in a few studies (Wang et al., 2008; Wei et al., 2015). The fluid-phase membrane is necessary to support membrane proteins and to regulate molecular transport into and out of the cell (Lingwood and Simons, 2010). However, very limited types of NPs have been investigated using model membranes. The NP content, surface chemistry and physiochemical properties may relate to the extent of the induced membrane disruption and cytotoxicity in general. Due to the abundant types of existing nanomaterials and the fast occurrence of new groups of NPs, further studies are still in need to expand our knowledge for the NP-membrane interaction.

Al_2O_3, Fe_2O_3, SiO_2, TiO_2 and ZnO NPs are the most common engineered oxide NPs. Each type of NPs has unique physiochemical properties, possibly causes different influences to the membrane morphology and phase after exposure. The interactions of the above-mentioned oxide NPs with membranes are important to predict the safety of nanomaterials. Therefore, this study was designed to investigate the effects of the five oxide NPs on membrane integrity and fluidity. The phospholipid molecular damage was also evaluated by Fourier transform infrared (FT-IR) analysis.

1. Materials and method

1.1. Materials

Al_2O_3, SiO_2, TiO_2 and ZnO NPs were purchased from Zhejiang Hongsheng Material Technology Co., China. The Fe_2O_3 NPs used in this study were single-crystal α-Fe_2O_3 nanorings prepared by a hydrothermal method using $FeCl_3$ and additives (Jia et al., 2008). Their morphology was controlled by the ratios of phosphate and sulfate ions to ferric ions in the nanostructure formation. The phospholipids 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-[phospho-*rac*-(1-glycerol) (sodium salt) (DOPG), 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) (DOTAP), and fluorescent lipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (RhB-PE) were all purchased from Avanti Polar Lipids (Alabaster, AL, USA). 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan) were purchased from Molecular Probes (Eugene, OR, USA).

1.2. Vesicle preparation

Gentle hydration method was used to prepare GUVs as mentioned by Akashi (Akashi et al., 1996). First, 18 mg/mL DOPC, 2 mg/mL DOPG and 2 mg/mL DOTAP solutions were prepared by dissolving phospholipids into chloroform:methanol (2:1 by volume). DOPG is negatively charged and DOTAP is positively charged lipid. Therefore, 50 μL DOPG or DOTAP was mixed with 50 μL DOPC solution to make the negatively charged ($GUVs^-$) or positively charged vesicles ($GUVs^+$), respectively. To prepare the fluorescent vesicles, 0.1% (W/W) RhB-PE was added to the lipid mixture. Then 100 μL lipid mixture was evaporated to form a thin lipid film under N_2 gas in a glass tube. The organic solvent residues in lipid film were removed in vacuum for more than 2 hr. Next the tube was filled by 0.1 mol/L sucrose and was incubated at 37°C for 24 hr to form GUVs. GUVs were collected and subsequently diluted into 0.1 mol/L glucose. The compositions and structures of GUV^-, GUV^+ and the fluorescent GUV were illustrated in Fig. 1.

To prepare small unilamellar vesicles (SUVs), same lipid solution was used as GUV preparation. After the lipid film was prepared on the inner surface of glass tube, the film was incubated in DI water at 37°C for 1-hr gentle hydration, and then the solution was pushed to pass through a 100-nm pore polycarbonate filter in an extruder for more than 25 times (Papadia et al., 2016).

1.3. Characterization of the oxide NPs

The zeta potentials of GUVs and oxide NPs were measured by a Malvern zeta-sizer (Malvern Instruments, Worcs, UK). Each sample was measured in DI water and 0.1 mol/L glucose at pH 6.5. The hydrodynamic diameter (*d*H) values of oxide NPs were measured through dynamic light scattering in 0.1 mol/L glucose. The morphology and diameters of single particles were investigated by transmission electron microscopy (TEM, JEM-1011, JEOL, Japan) at the electron emission of 100 kV and the images were shown in Fig. 2. The physiochemical properties of oxide NPs are listed in Table 1.

1.4. Interaction between oxide NPs and GUVs

NP suspension was added into GUV solution in 0.1 mol/L glucose in a glass bottom (0.15 mm thickness) Petri dish to achieve proper concentration. After exposure of 10 min, 40 min, 90 min and 24 hr, GUVs were imaged using an inverted microscope with 40× objective lenses. The different refractive indices of inside sucrose and external glucose make GUVs visible under bright field. For fluorescent imaging, RhB-labeled GUVs were imaged by Carl Zeiss LSM 700 fluorescence confocal microscope.

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