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Dispersion of atmospheric fine particulate matters in simulated lung fluid and their effects on model cell membranes

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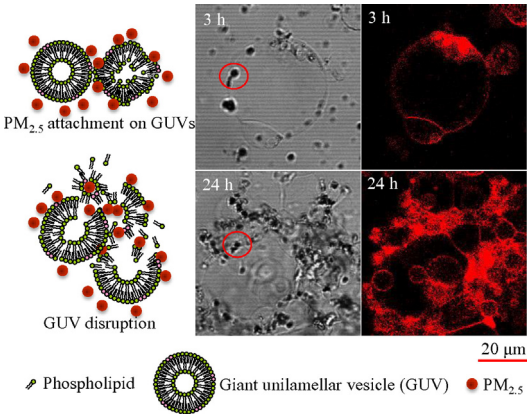
HIGHLIGHTS

- Proteins disperse $PM_{2.5}$ in lung fluid and decrease its hydrodynamic diameter.
- Phospholipids cause aggregation and fast sedimentation of $PM_{2.5}$.
- $PM_{2.5}$ adheres on the cationic sites and disrupts model cell membranes.

GRAPHICAL ABSTRACT



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ABSTRACT

Atmospheric fine particulate matter ($PM_{2.5}$) was collected to investigate its dispersion in simulated lung fluid (SLF) and its interaction with model cell membranes. Organic acids, NH_4^+, SO_4^{2-} and NO_3^- were detected in $PM_{2.5}$ soluble fraction, and heavy metals were detected from the total mass. The insoluble fraction contained kaolinite, $CaCO_3$, aliphatic carbons, aromatic rings, carboxyl and hydroxyl groups reflected by the infrared spectra. Proteins dispersed $PM_{2.5}$ in SLF, resulted in smaller hydrodynamic diameter (d_H) and slower sedimentation rate. Conversely, phospholipids increased d_H value and accelerated sedimentation rate. Giant unilamellar vesicles (GUVs) and supported lipid bilayers (SLBs) were used as model cell membranes. $PM_{2.5}$ adhered on and disrupted the membrane containing positively-charged lipids but not the membrane containing neutrally- and negatively-charged lipids, which was monitored by microscopy and a quartz crystal microbalance with dissipation (QCM-D). The cationic sites on membrane were necessary for $PM_{2.5}$ adhesion, but membrane should be disrupted by the combined action of electrostatic forces and hydrogen bonds between $PM_{2.5}$ oxygen containing groups and the lipid phosphate groups. Our results specified the roles of proteins and phospholipids in $PM_{2.5}$ dispersion and transport, highly suggested that the health hazard of $PM_{2.5}$ was related to the biomolecules in the lung fluid and the particle surface groups.

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1. Introduction

Recent years, frequent outbreaks of haze have attracted much public attention in China (Ma et al., 2012; Zhang et al., 2012). The formation of haze and the components of atmospheric particulate matters (PM) have been widely explored (Chen and Xie, 2014; Ji et al., 2014). Long-term exposure to PM leads to high mortality for people with lung, cardiovascular or metabolic diseases (Kim et al., 2015; Laing et al., 2010; To et al., 2015), increases mortality in the elderly (Cakmak et al., 2011), and slows the lung function development in children (Gauderman et al., 2004). In pulmonary system, PM has been reported to induce inflammatory reactions (Liu et al., 2014), penetrate the alveolar epithelium (Kampa and Castanas; 2008) and cause endoplasmic reticulum stress (Laing et al., 2010).

The size of PM is one of the key factors controlling their transport and toxicity in body (Farina et al., 2011). Atmospheric fine particulate matters ($PM_{2.5}$, aerodynamic diameters ≤ 2.5 μm) can pass the protective barriers such as mucociliary system, arrive at alveoli and enter lung fluid (Kim et al., 2015). $PM_{2.5}$ has been reported to aggregate in lung fluid, which is rich of phospholipids and proteins so called lung surfactant (Kendall et al., 2002). Smaller particles have higher chance to pass the air–blood barrier and reach remote body organs (Kreyling et al., 2014; Kim et al., 2015), and faster release of harmful contents such as heavy metals and polycyclic aromatic hydrocarbons (PAHs) (Fang et al., 2013; Kampa and Castanas, 2008; Zereini et al., 2012). $PM_{2.5}$ adsorbs phospholipids and proteins in alveoli (Kendall et al., 2004; Kendall, 2007), but the roles of phospholipids and proteins in $PM_{2.5}$ aggregation and dispersion are unclear. Moreover, although $PM_{2.5}$ samples are collected by their aerodynamic diameters, their hydrodynamic diameter (d_H) in lung fluid and size distribution in the presence or absence of phospholipids/proteins have not been studied.

The inhaled $PM_{2.5}$ interacts with alveolar epithelial cells and macrophages, which possibly induce pulmonary fibrosis (Dysart et al., 2014), oxidative damage or even cell apoptotic (Deng et al., 2014; Orona et al., 2014). The interaction between particles and cell membranes is the first step for particle internalization and inducing cytotoxicity (Chen and Bothun, 2014). Cell membrane is the crucial structure to maintain the independent intracellular environment, to regulate the exchange of materials inside and outside of cell, to transmit signal, and to recognize cell types (Pontes et al., 2013; Wei et al., 2015). PM has been reported to affect cell membrane integrity, induce hemolysis and pro-inflammatory effects (Geng et al., 2005; Osornio-Vargas et al., 2011). PM composition and surface characteristics are suggested to dominate the interaction between particles and cell membrane, resulting in membrane leakage and lipid fluidity alteration (Geng et al., 2005; Geng et al., 2006). However, the mechanisms of $PM_{2.5}$-membrane interaction and membrane disruption are largely unknown. Roles of electrostatic forces and other intermolecular forces in this process still need to be further evaluated and discussed. Giant unilamellar vesicles (GUVs) and supported lipid bilayers (SLBs) have similar composition and structure compared with real cell membranes, which are widely used to mimic cellular membranes and exclude the uncertainties of cell physiological activities (Laurencin et al., 2010; Wei et al., 2015).

Therefore, this research aims to study $PM_{2.5}$ dispersion in lung fluid and its interaction with cell membranes, and to better understand $PM_{2.5}$ alveolar transport and its cytotoxicity. Hence, effects of proteins and phospholipids on d_H and deposition of $PM_{2.5}$ were evaluated in simulated lung fluid (SLF). The $PM_{2.5}$ attachment on membranes and induced membrane disruption were monitored using GUVs and SLBs. Roles of electrostatic force and $PM_{2.5}$ surface functional groups were discussed based on the analysis of $PM_{2.5}$ composition and physiochemical properties.

2. Materials and methods

2.1 Materials

Phospholipids 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (sodium salt) (DOPG), 1,2-dipalmitoyl-3-trimethylammonium-propane (chloride salt) (16:0 TAP), 1,2-dipalmitoyl-sn-glycero-3-phosphochollne (DPPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (RhB-PE) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Bovine serum albumin (BSA) was purchased from Sigma-Aldrich. Glucose, sucrose and inorganic salts were obtained from Kermel (China). Tris-(hydroxymethyl)-aminomethane (Tris) was supplied by Amresco (Solon, OH, USA).

2.2 $PM_{2.5}$ sample collection and characterization

Atmospheric fine particulate matters were collected from December 2014 to February 2015 in the observation station located in the campus of Shandong University, which is an urban site in the city of Jinan (36.67° N, 117.05° E). The samples were gathered using Isopore™ membrane filters (90-mm diameter, 0.4 μm HTTP, Merck Millipore ltd., Germany) at a flow rate of 100 L/min by a medium-volume $PM_{2.5}$ sampler. All the filters were sonicated to dissolve or disperse $PM_{2.5}$ samples into deionization (DI) water to separate $PM_{2.5}$ from filters. Then $PM_{2.5}$ was re-collected by freeze-drying. A small part of suspension was centrifuged at 1780 g for 20 min to discard the supernatant before freeze-drying and to obtain the insoluble fraction. Additional paralleled samples were collected by a medium-volume $PM_{2.5}$ sampler (100 L/min) with quartz filters for $PM_{2.5}$ composition analysis.

The daily concentrations of organic acids (acetic, formic, lactic, methane sulfonic acids) and water-soluble ions (Cl^-, NO_3^-, SO_4^{2-}, Na^+, NH_4^+, Mg^{2+} and Ca^{2+}) were determined by an ion chromatography (IC, model Dionex 2500). The organic carbon (OC) and elemental carbon (EC) fractions were measure by a thermal–optical transmission carbon analyzer (Sunset Laboratory Inc., USA). The heavy metals in the total collected $PM_{2.5}$ sample were measured after acid digestion (HNO_3: HCl = 1:3) at 100 °C for 2 h. Cd, Co, Cr, Cu, Mn, Ni, Pb, Sb, Sn and Zn were measured by an inductively coupled plasma mass spectrometry (ICP-MS, iCAPQC, Thermo Fisher, USA). As was measured by an atomic fluorescence spectrometer (AFS-920, Beijing Titan Instruments, China), and Fe was measured by an ICP optical emission spectrometer (ICP-OES, Optima 7000DV, Perkin Blmer, USA). Fourier transform infrared (FTIR) spectra of the total collected $PM_{2.5}$ sample and its insoluble fraction were obtained through an IR spectrometer (NEXUS 670, Thermo Nicolet, Germany) to identify the functional groups at 2 cm^{-1} resolution and more than 50 scans.

2.3 Preparation of simulated lung fluid

Since it is hard to obtain and store alveolar fluid, SLF is wildly used to simulate the interstitial fluid in the deep lung (Colombo et al., 2008; Zereini et al., 2012). SLF was prepared based on the component of Gamble's solution which include 0.203 g/L $MgCl_2 \cdot 6H_2O$, 6.091 g/L NaCl, 0.298 g/L KCl, 0.318 g/L $Na_2HPO_4 \cdot 12H_2O$, 0.143 g/L $Na_2SO_4 \cdot 10H_2O$, 0.278 g/L $CaCl_2$, 0.952 g/L $NaC_2H_3O_2 \cdot 3H_2O$ and 2.604 g/L $NaHCO_3$ (Colombo et al., 2008). $C_6H_5Na_3O_7$ which presented proteins in Gamble's solution was not included because the role of proteins was explored individually by adding 0.2 mM BSA (SLF + BSA). Similarly 0.2 mg/L DPPC was added to SLF (SLF + DPPC) to study the effect of phospholipids.