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Ultrafine particle libraries for exploring mechanisms of $PM_{2.5}$-induced toxicity in human cells

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ABSTRACT

Air pollution worldwide, especially in China and India, has caused serious health issues. Because $PM_{2.5}$ particles consist of solid particles of diverse properties with payloads of inorganic, organic and biological pollutants, it is still not known what the major toxic components are and how these components induce toxicities. To explore this complex issue, we apply reductionism principle and an ultrafine particle library approach in this work. From investigation of 63 diversely functionalized ultrafine particles (FUPs) with adsorbed key pollutants, our findings indicate that 1) only certain pollutants in the payloads of $PM_{2.5}$ are responsible for causing cellular oxidative stress, cell apoptosis, and cytotoxicity while the particle carriers are much less toxic; 2) pollutant-induced cellular oxidative stress and oxidative stress-triggered apoptosis are identified as one of the dominant mechanisms for $PM_{2.5}$-induced cytotoxicity; 3) each specific toxic component on $PM_{2.5}$ (such as As, Pb, Cr or BaP) mainly affects its specific target organ(s) and, adding together, these pollutants may cause synergistic or just additive effects. Our findings demonstrate that reductionism concept and model $PM_{2.5}$ particle library approach are very effective in our endeavor to search for a better understanding of $PM_{2.5}$-induced health effects.

1. Introduction

Air pollution causes nearly 8% of all deaths wordwide (Cohen et al., 2017), among which half are from China and India. Long-term exposures to polluted air is also linked to diseases, such as respiratory infections (Cruz-Sanchez et al., 2013), stroke (O'Donnell et al., 2011), chronic obstructive pulmonary disease (Anderson et al., 1997; Sunyer, 2001), heart attack (Vera et al., 2009), and cancer (Chalbot et al., 2014; Hu et al., 2014; Morita et al., 2014). It is highly imperative to identify key toxic components in air-borne $PM_{2.5}$ and understand the associated mechanisms. In the highly polluted Chinese cities, Baoding has been among the top two (http://www.cnemc.cn/). Shanghai and Guangzhou, like many Chinese cities, also have bad air quality (Liu et al., 2014, 2017; Song et al., 2017; Wu et al., 2017). Due to different industrial portfolio in these cities, $PM_{2.5}$ from Baoding, Shanghai and Guangzhou have different pollution dynamics and pollutants profiles. Furthermore, components of $PM_{2.5}$ also vary with the times of the day and the seasons of the year (Song et al., 2017). Therefore, it is a formidable task to mimic $PM_{2.5}$ and elucidate the toxicity mechanisms of these harmful particles. To tackle this difficult problem, here we are applying the reductionism principle and taking a systematic ultrafine particle library approach.

$PM_{2.5}$ particles adsorb organic compounds, inorganic metal ions, or biological contaminants (Yang et al., 2011; Li et al., 2013; Huang et al., 2014; Kim et al., 2015). $PM_{2.5}$ particles reduce their sizes in water because soluble inorganic salts components (such as $((NH_4)_2SO_4$, KNO_3…) of $PM_{2.5}$ are soluble, while insoluble parts (such as black carbon) with adsorbed pollutants remain as small particles in water. We found that soluble components of $PM_{2.5}$ in general exhibited much less cytotoxicity to normal human lung and kidney cells compared to insoluble particles (Fig. S1). Complex components make the insoluble $PM_{2.5}$ particles possess diverse hydrophobicity, electric charge, and various chemical reactivities or toxicities. To model these particles and reveal toxicity induction mechanisms, we synthesized model FUP particles of various physicochemical properties with diverse payloads of pollutants. To validate this reductionism approach, here we investigated 63 representative FUPs with adsorption of controlled amounts of pollutants founds in $PM_{2.5}$. Although there are many

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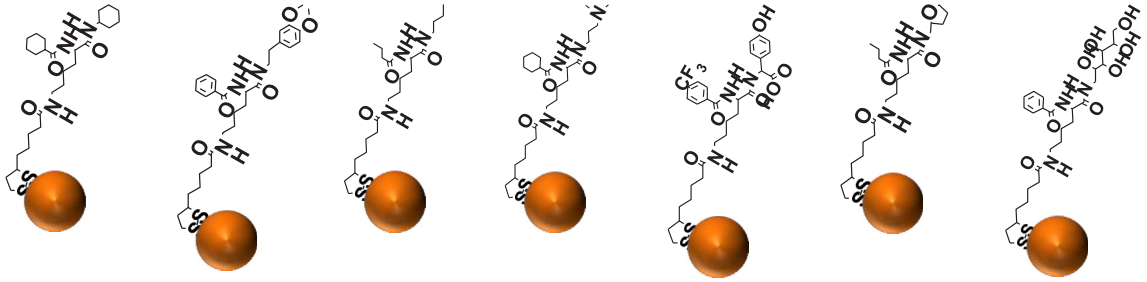
Table 1
Chemical structures of 63 FUPs.



Blank	FUP**1**	FUP**2**	FUP**3**	FUP**4**	FUP**5**	FUP**6**	FUP**7**
BaP	FUP**1**-BaP	FUP**2**-BaP	FUP**3**-BaP	FUP**4**-BaP	FUP**5**-BaP	FUP**6**-BaP	FUP**7**-BaP
As (III)	FUP**1**-As	FUP**2**-As	FUP**3**-As	FUP**4**-As	FUP**5**-As	FUP**6**-As	FUP**7**-As
Pb^{2+}	FUP**1**-Pb	FUP**2**-Pb	FUP**3**-Pb	FUP**4**-Pb	FUP**5**-Pb	FUP**6**-Pb	FUP**7**-Pb
Cr (VI)	FUP**1**-Cr	FUP**2**-Cr	FUP**3**-Cr	FUP**4**-Cr	FUP**5**-Cr	FUP**6**-Cr	FUP**7**-Cr
As (III) + BaP	FUP**1**-As- BaP	FUP**2**-As- BaP	FUP**3**-As- BaP	FUP**4**-As- BaP	FUP**5**-As- BaP	FUP**6**-As- BaP	FUP**7**-As- BaP
Pb^{2+} + BaP	FUP**1**-Pb- BaP	FUP**2**-Pb- BaP	FUP**3**-Pb- BaP	FUP**4**-Pb- BaP	FUP**5**-Pb- BaP	FUP**6**-Pb- BaP	FUP**7**-Pb- BaP
Cr(VI) + BaP	FUP**1**-Cr- BaP	FUP**2**-Cr-BaP	FUP**3**-Cr- BaP	FUP**4**-Cr- BaP	FUP**5**-Cr- BaP	FUP**6**-Cr- BaP	FUP**7**-Cr- BaP
As (III) + Pb^{2+} + Cr(VI) + BaP	FUP**1**-As- Pb-Cr -BaP	FUP**2**-As-Pb-Cr-BaP	FUP**3**-As- Pb-Cr-BaP	FUP**4**-As- Pb-Cr-BaP	FUP**5**-As- Pb-Cr-BaP	FUP**6**-As- Pb-Cr-BaP	FUP**7**-As-Pb-Cr-BaP

components in $PM_{2.5}$, we selected the most toxic pollutants As(III), Pb^{2+}, Cr(VI) and BaP as representative pollutants because they can induce serious damage to human health in a very low concentration (Jin et al., 2016).

2. Materials and methods

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and were used without purification.

2.1. $PM_{2.5}$ collection, preparation and characterization

$PM_{2.5}$ particles were collected at cities of Baoding, Shanghai and Guangzhou. $PM_{2.5}$ was collected daily on Whatman quartz fibre filters, using a TH-1000 Ambient Particulate Sampler (Tianhong Instruments, Wuhan, China), at a flow rate of 1050 L/min. After sampling, the filters were stored in the dark at − 18 °C. Each filter was sonicated for 1 h in 150 mL ultrapure H_2O in ice bath. The water-soluble fraction was removed and the black particles on the filter collected gently. Then the filter sonicated for 1 h in 100 mL dimethyl sulfoxide (DMSO) in ice bath and repeated the steps. Finally all the extract and black particle were combined and dried in a vacuum freeze dryer.

Components in $PM_{2.5}$ were analyzed by an array of techniques. Water-soluble ionic species were analyzed using ionic chromatography (Dionex ICS-3000), inorganic metal elements, by inductively coupled plasma-mass spectrometry, organic carbon/Elemental carbon (OC/EC), by Thermal/Optical Carbon Analyzer (Desert Research Institute, DRI Model 2001A) and PAHs, by gas chromatography-mass spectrometry (Agilent, GC-MS, 7890 A/5975 C).

2.2. General procedure for synthesis of FUPs (Scheme S1)

The synthesis of diverse organic ligands TA-Lys-R_1R_2 is described in SI. TA-Lys-R_1R_2 (0.064 mmol) was dissolved in 20 mL dimethylformamide (DMF). Gold(III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$) was added. After stirring for 30 min at room temperature, ice-cold sodium tetrahydroborate solution in (12 mL, 15.8 mM) was dropwise added to the mixture. The solution turned red immediately and was stirred for 4 h at room temperature. The solution was centrifuged at 15,000 rpm for 30 min. The precipitation was washed with the mixture solution of DMF and H_2O (1:1 (v/v)) for 4 times to remove the free ligands. Subsequently, the product was washed with H_2O for 3 times to remove the DMF, and dispersed in H_2O as stock solution.

2.3. Characterization

Before and after sonication (30 min, 0.04 M Tween 20 solution), the morphology and sizes of $PM_{2.5}$ and FUP particles were analyzed by transmission electron microscope (TEM) (JEM-1011, JEOL). Dynamic hydrodynamic diameters and zeta potentials of FUPs and $PM_{2.5}$ solutions (40 μg/mL) in water or cell culture medium with 10% serum were measured by dynamic light scattering (DLS) (Malvern Nano Z Zetasizer, Malvern Instruments). Modified “shaking flask” method was employed to measure the LogP of particles, which was reported in our previous work (Li et al., 2015).

2.4. Cell culture

HEK293 and 16HBE cells were cultured in Dulbecco's modified Eagle's medium (DMEM) or minimum essential medium (MEM), respectively, supplemented 10% fetal bovine serum (FBS), 10 U/mL penicillin, and 10 mg/mL streptomycin. Cultures were maintained in a 37 °C incubator with 95% humidity and 5% CO_2. Cells used in the experiments were collected in the logarithmic phase.

2.5. Cell viability measurements

HEK293 and 16HBE cells were seeded in a 96-well plate with the density of 7000 cells per well. After incubation for 24 h, medium in each well were replaced with fresh cell culture medium containing different amounts of FUPs or $PM_{2.5}$. Cells were incubated with particles for 24 h. The percentage of viable cells in each well was then determined by CellTiter-Glo assay (Promega Corporation, USA) as described in the manufacture's protocol. The mass ratio of BaP (0.1%), As (III) (0.1%), Pb^{2+} (0.5%) and Cr (VI) (0.1%) in FUPs were in the range of their amounts as detected in $PM_{2.5}$ particles.

2.6. Determination of cellular oxidative stress

HEK293 and 16HBE cells were seeded in a 24-well plate (50,000 cells per well). After incubation for 24 h, the medium in each well were removed. Solutions of FUPs, pollutant-loaded FUPs or $PM_{2.5}$ in cell culture medium (100 μg/mL) were added and incubated with cells for 24 h. The cells were then washed with PBS twice and 2,7-