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Toxicological Effects of Artificial Fine Particulate Matter in Rats through Induction of Oxidative Stress and Inflammation

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Airborne fine particulate matter with an aerodynamic diameter equal to or smaller than 2.5 μm (abbreviated as $PM_{2.5}$) increases the risk of nasal lesions, but the underlying molecular mechanism has not been fully elucidated. In the atmosphere, the composition of $PM_{2.5}$ collected varies in physical and chemical properties, which affects its damage to human health. Thus, we constructed artificial $PM_{2.5}$ particles based on actual $PM_{2.5}$ and investigated the in vivo effects of artificial $PM_{2.5}$ exposure on the oxidative stress, inflammatory response, and nasal mucosa morphology of rats. The results showed that artificial $PM_{2.5}$ is comparable in composition ratio, size, and morphology to actual $PM_{2.5}$. This in vivo study indicated that artificial $PM_{2.5}$ exposure reduces total superoxide dismutase and glutathione peroxidase activities, elevates malondialdehyde content in the nasal mucosa, and induces increased levels of pro-inflammatory mediators, including interleukin-1, interleukin-6 and tumor necrosis factor-α. Our data shows that artificial $PM_{2.5}$ particles could be used for experimental study of $PM_{2.5}$ toxicology, ensuring that the physical and chemical properties of experimental $PM_{2.5}$ are relatively constant and allowing for repeatability of this research. Oxidative damage and inflammatory response may be the toxic mechanisms that cause nasal lesions after exposure to artificial $PM_{2.5}$.

Keywords: glutathione peroxidase; interleukin-1; $PM_{2.5}$; total superoxide dismutase; tumor necrosis factor-α
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Introduction

$PM_{2.5}$ is a group of particulate matter (PM) with an aerodynamic diameter equal to or smaller than 2.5 μm. $PM_{2.5}$-induced public health hazards have attracted increasing attention, especially in developing countries such as China (Zhang et al. 2018; Lin et al. 2018). Epidemiological studies have found that $PM_{2.5}$ exposure increases the risk of ischemic heart disease, stroke (Hayes et al. 2020) and respiratory diseases (Pun et al. 2017). The nose is a natural pathway for breathing and a major target for many inhaled toxicants in air pollution, including $PM_{2.5}$. Exposure to $PM_{2.5}$ increases the risk of nasal lesions, and a damaged nasal mucous membrane will produce discharge, congestion, and swelling, and leads to loss of the barrier integrity of the human nasal epithelial (Xian et al. 2020). The molecular mechanism for its toxicity has yet to be fully elucidated. Toxicological studies of ambient $PM_{2.5}$ are required to understand its harmful effects on nasal passageways.

The various effects of $PM_{2.5}$, such as air transport capacity, air visibility and damage to human health, are

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dependent on its physical properties, including shape, surface characteristics, mass concentration, and particle size distribution. $PM_{2.5}$-induced damage to human health is also affected by its chemical properties. At present, most of the toxicological studies on $PM_{2.5}$ have used $PM_{2.5}$ collected from the atmosphere (Yan et al. 2014; Li et al. 2015; Zhang et al. 2015), the composition of which varies by region, season, and time of day. With this lack of a constant $PM_{2.5}$ composition, the repeatability of studies is difficult. Finding a method to maintain the repeatability of $PM_{2.5}$ toxicology research is of great significance to this field. The creation of artificial $PM_{2.5}$ particles that match the characteristics of atmospheric $PM_{2.5}$ would allow for $PM_{2.5}$ with relatively stable physical and chemical properties to be used for experimental study.

Oxidative stress, a state of imbalance between oxidation and antioxidation, plays an important role in the pathology of many human diseases (Limon-Pacheco and Gonsebatt 2009; Mudway et al. 2020). Previous studies have suggested that $PM_{2.5}$ has the potential to stimulate cells to produce and release inflammatory cytokines (Nagaoka et al. 2019). Inhaled $PM_{2.5}$ makes contact with the nasal mucosa, and initiates and augments local inflammation, which might impair nasal epithelial barrier function, resulting in the prevalence of nasal inflammatory diseases such as chronic and allergic rhinitis. In conjunction with allergens, $PM_{2.5}$ acts as an adjuvant, showing additive effects on allergic rhinitis (Zhang et al. 2015). In vitro studies have found that oxidative stress and the inflammatory response play an important role in the nasal epithelium, contribute to the impairment of the nasal epithelial barrier following $PM_{2.5}$ exposure, and further decrease cell viability (Hong et al. 2016). However, the underlying molecular mechanism is largely unknown, especially because of the lack of in vivo toxicological studies.

In this study, actual $PM_{2.5}$ was collected in Shanghai, China, followed by the assessment of its size and composition. We constructed artificial $PM_{2.5}$ particles based on the size and composition of actual $PM_{2.5}$. These artificial $PM_{2.5}$ particles simulate the physical and chemical characteristics of the actual $PM_{2.5}$. Using these artificial $PM_{2.5}$, we created an inhalation exposure animal model to conduct toxicological studies in vivo and provide the experimental basis and technical platform for studying artificial $PM_{2.5}$ damage and its underlying mechanism. We hypothesized that the nasal mucosa plays a key role in nasal inflammatory diseases by oxidative stress and producing numerous inflammatory cytokines. The present study aimed to examine the effects of artificial $PM_{2.5}$ by estimating nasal oxidative stress responses and inflammation in rats.

Materials and Methods

Actual $PM_{2.5}$ analysis and artificial $PM_{2.5}$ preparation

The actual $PM_{2.5}$ was collected every day for four months (January, April, July and October) in 2013 at Fudan University (31.3°N, 121.5°E) in Shanghai, China. Physical and chemical characterization of actual PM2.5 was determined as previously described (Hong et al. 2016). Inductively coupled plasma atomic emission spectroscopy (ICP-OES; SPECTRO, Kleve, Germany) was used to measure eleven metals (Al, As, Ca, Fe, K, Mg Na, Pb, S, Ti and Zn). Six inorganic ions (NO_3^-, SO_4^{2-}, NH_4^+, Ca^{2+}, Cl^- and Na^+) were analyzed by ICS 3000 ion chromatography (Dionex, Sunnyvale, CA, USA).

We constructed artificial $PM_{2.5}$ particles based on the physical and chemical properties of actual $PM_{2.5}$. Activated carbon was used as the core of artificial $PM_{2.5}$. Chemical species were loaded onto the pores of the activated carbon using the impregnation method. According to the proportion of actual $PM_{2.5}$ components, artificial $PM_{2.5}$ components comprise activated carbon (2%), CaC_2O_4 (14%), NH_4HSO_4 (20%), NH_4NO_3 (24%), NaCl (5%), SiO_2 (1.06%), S (17.69%), Ca (5.03%), Fe (3.06%), Na (2.37%), Al (2.26%), K (1.29%), Mg (0.80%), Zn (0.58%), Ti (0.31%), As (0.19%), Pb (0.18%) and polycyclic aromatic hydrocarbons (PAHs; 0.18%). All artificial $PM_{2.5}$ components mentioned above were ground using a DQM-0.4L ball mill (Qile Electronic Technology Co. Ltd., Jiangsu, China) for at least 15 minutes and added to an ethanol solution. Magnetic stirring was performed at room temperature for 24 hours to maximize the loading efficiency. Then, the activated carbon loaded with chemical species was collected via centrifugation, washed twice with ethanol, and dried under a vacuum. Scanning electron microscopy S4800 (SEM; Hitachi, Tokyo, Japan) was used to determine the size and morphology of the actual $PM_{2.5}$ and artificial $PM_{2.5}$.

Animals and $PM_{2.5}$ exposure protocol

The experiments were carried out with female Sprague–Dawley rats (4-5 weeks old, 210-240 g weight), which were specific pathogen-free (SPF) and purchased from the Experimental Animal Center of Fudan University (Shanghai, China). The animals were housed in Makrolon cages with a 12-h light-dark cycle. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Fudan University.

Thirty-two Sprague–Dawley rats were assigned to four equal groups matched with respect to age and weight: (1) negative control (NC) group; (2) a low concentration of artificial $PM_{2.5}$ exposure ($LarPM_{2.5}$) group, exposed to 200 $\mu g/m^3$ artificial $PM_{2.5}$; (3) a moderate concentration of artificial $PM_{2.5}$ exposure ($MarPM_{2.5}$) group, exposed to 1,000 $\mu g/m^3$ artificial $PM_{2.5}$; (4) a high concentration of artificial $PM_{2.5}$ exposure ($HarPM_{2.5}$) group, exposed to 3,000 $\mu g/m^3$ artificial $PM_{2.5}$. The $PM_{2.5}$ inhalation exposure system includes sealed transparent exposure observation chamber, $PM_{2.5}$ aerosol generation system, $PM_{2.5}$ monitoring system, air input and output system and $PM_{2.5}$ filtering system. The $PM_{2.5}$ aerosol generator injects a certain amount of artificial $PM_{2.5}$ aerosol into the exposure chamber and mixes it with a certain amount of diluted air. The $PM_{2.5}$ monitoring system

can monitor the $PM_{2.5}$ concentration in the exposure chamber in real time. The $PM_{2.5}$ filter is used to filter the exhaust gas to avoid polluting the environment (Guo et al. 2017).

Rats were exposed to $PM_{2.5}$ in a quadrangular chamber ($70 \times 55 \times 45$ cm^3) connected to air pumps (HSENG AS18-2, Beijing, China) with a liquid aerosol generator (HRH-WAG6, Beijing, China), which produced particles with aerodynamic diameters less than 2.5 μm. The particle concentration was measured using a $PM_{2.5}$ detector (PC-3A, Jiangsu, China). A high-efficiency particulate air (HEPA) filter was placed at the outlet of the chamber designated for inside–outside air exchange. The addition of a HEPA filter prevented the $PM_{2.5}$ from exiting the chamber. The NC group was exposed to saline for 3 h/day for 30 consecutive days from Day 0 to Day 29. The other three groups of animals were exposed to different concentrations of $PM_{2.5}$ for 3 h/day for 30 consecutive days.

Histological evaluation

The rats were euthanized using pentobarbital sodium (30 mg/kg·bw, i.p.) 24 h after the last inhalation of $PM_{2.5}$. The nasal septum mucosa was harvested and fixed in 10% formaldehyde then processed routinely, embedded in paraffin, sectioned into 4-μm-thick sections, and stained with hematoxylin and eosin (HE). The histopathological changes were evaluated in tissue sections with an Olympus BX40 microscope.

Antioxidant enzyme assays

Nasal tissue proteins were extracted with a protein extraction kit (Beyotime, Shanghai, China). The levels of total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were measured by superoxide dismutase, glutathione peroxidase and malondialdehyde assay kits according to manufacturer's instructions (Beyotime). Total protein concentration was determined by a bicinchoninic acid protein assay kit (Beyotime), and enzyme activity was standardized to milligram protein.

Enzyme-linked immunosorbent assay (ELISA)

Blood samples were collected from the abdominal aorta. Serum samples were prepared after incubation in ice-temperature storage and centrifugation at $1{,}800 \times g$ for 15 min and were stored at −80°C until analysis. The levels of interleukin-1 (IL-1), IL-6 and tumor necrosis factor-α (TNF-α) in the serum of the tested rats were analyzed with an enzyme-linked immunosorbent assay (ELISA) using a commercial kit according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).

Statistics

Statistical analysis was performed using SPSS software for Windows (version 25.0; SPSS, Inc., Chicago, IL, USA). The results are expressed as the mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA). A P value < 0.05 was considered statistically significant.

Results

Chemical characterization of actual $PM_{2.5}$

Metal and ion constituents of actual $PM_{2.5}$ were analyzed by ICP-OES and ICS. Table 1 shows the analytical results for 11 elements and 6 ions. The content of element S was highest, 11.92 ± 2.27 $\mu g/m^3$, followed by Ca (3.39 ± 1.14 $\mu g/m^3$), Fe (2.06 ± 0.98 $\mu g/m^3$), Na (1.60 ± 0.06 $\mu g/m^3$) and Al (1.52 ± 0.05 $\mu g/m^3$). Among the 6 ions analyzed, NO_3^- had the highest ion content, up to 16.12 ± 2.17 $\mu g/m^3$, followed by SO_4^{2-} (13.09 ± 1.36 $\mu g/m^3$), NH^{4+} (8.26 ± 0.87 $\mu g/m^3$), Cl^- (2.64 ± 0.17 $\mu g/m^3$) and Na^+ (1.02 ± 0.19 $\mu g/m^3$).

Construction of artificial $PM_{2.5}$ based on the features of actual $PM_{2.5}$

Artificial $PM_{2.5}$ particles ($arPM_{2.5}$) were prepared with activated carbon as the core and a number of chemical species loaded onto the pores of the core. Table 2 compares the compositions of artificial $PM_{2.5}$ and actual $PM_{2.5}$, showing that artificial $PM_{2.5}$ was close to the composition ratio of actual $PM_{2.5}$. The primary ion components were NO_3^-, SO_4^{2-}, NH_4^+ and a small amount of Ca^{2+}, Cl^- and Na^+. The primary elemental components were S, Ca, Fe, Na, Al and K. Sixteen priority PAHs of the Environmental Protection Agency (EPA) were relatively heavy in actual $PM_{2.5}$ content, indicating a potential for great harm to human health. In accordance with reports in the literature and the results of our study (Hong et al. 2016), 0.18% PAHs were added to artificial $PM_{2.5}$.

Scanning electron microscopy was used to determine the physical characteristics of actual $PM_{2.5}$ and artificial $PM_{2.5}$, such as size and morphology. Fig. 1 shows that particulate matter with a diameter equal or smaller than 2.5 μm was the most abundant in both actual $PM_{2.5}$ and artificial $PM_{2.5}$. Some morphological differences were observed between artificial $PM_{2.5}$ and actual $PM_{2.5}$, including spheres,

Table 1. Ion and element constituents of actual $PM_{2.5}$.

Element	Content in actual $PM_{2.5}$ ($\mu g/m^3$)	Ion	Content in actual $PM_{2.5}$ ($\mu g/m^3$)
S	11.92 ± 2.27	NO_3^-	16.12 ± 2.17
Ca	3.39 ± 1.14	SO_4^{2-}	13.09 ± 1.36
Fe	2.06 ± 0.98	NH_4^-	8.26 ± 0.87
Na	1.60 ± 0.06	Ca^{2+}	3.39 ± 0.29
Al	1.52 ± 0.05	Cl^-	2.64 ± 0.17
K	0.87 ± 0.09	Na^+	1.02 ± 0.19
Mg	0.54 ± 0.04		
Zn	0.39 ± 0.04		
Ti	0.21 ± 0.02		
As	0.13 ± 0.01		
Pb	0.12 ± 0.02		

Data are shown as mean ± SD.

Table 2. Comparison of compositions between artificial $PM_{2.5}$ and actual $PM_{2.5}$.

Compositions	Content in artificial $PM_{2.5}$(%)	Content in actual $PM_{2.5}$(%)	Compositions	Content in artificial $PM_{2.5}$(%)	Content in actual $PM_{2.5}$(%)
S	17.69	17.69	NO_3^-	18.6	23.92
Ca	5.03	5.03	SO_4^{2-}	16.87	19.42
Fe	3.06	3.06	NH_4^+	8.53	12.26
Na	2.37	2.37	Ca_2^+	4.38	5.03
Al	2.26	2.26	Cl^-	3.02	3.92
K	1.29	1.29	Na^+	1.97	1.51
Mg	0.80	0.80	PAHs	0.18	0.18
Zn	0.58	0.58			
Ti	0.31	0.31			
As	0.19	0.19			
Pb	0.18	0.18			

PAHs, polycyclic aromatic hydrocarbons.

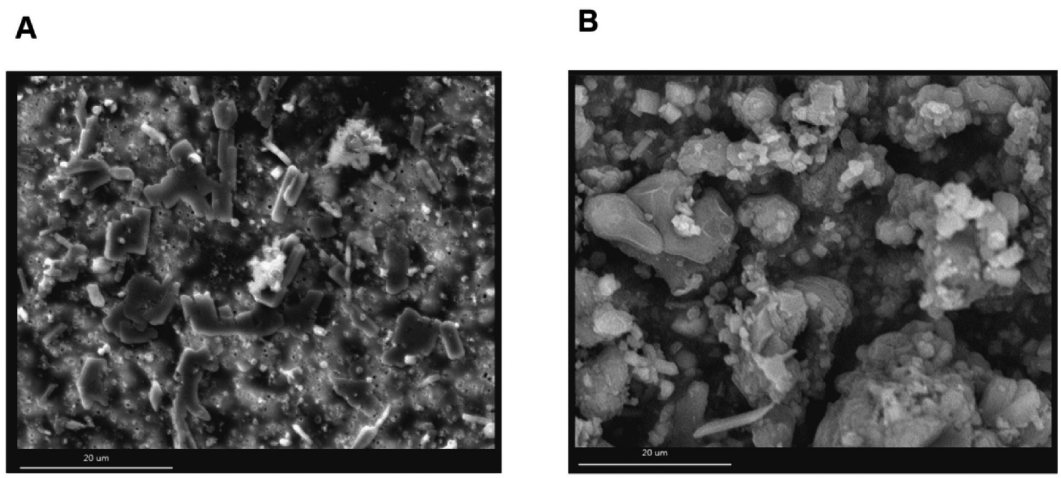

Fig. 1. Scanning electron microscopy observation of actual $PM_{2.5}$ particles (A) and artificial $PM_{2.5}$ particles (B). Original magnification: A, B × 20,000.

clusters, plate and reticular forms. Some particles were aggregated into a group, and some were in the form of a single particle.

Artificial $PM_{2.5}$-induced nasal mucosa histology damage

HE staining was used to observe the pathological changes of nasal mucosa after artificial $PM_{2.5}$ inhalation. The nasal mucosa from the NC group showed no histopathological abnormalities in HE staining, but different concentrations of artificial $PM_{2.5}$ exposure induced morphological alterations, including nasal mucosa epithelium disorder, increased submucosal infiltration of inflammatory cells, and even cell exfoliation (Fig. 2). As the $PM_{2.5}$ concentration increased, the nasal mucosa pathological injuries markedly increased.

Artificial $PM_{2.5}$-induced oxidative stress in the nasal mucosa

To assess whether artificial $PM_{2.5}$ induces oxidative stress, the levels of total T-SOD, GSH-Px and MDA were measured. Compared to the control group (NC), artificial $PM_{2.5}$ at concentrations of 200, 1,000, and 3,000 $\mu g/m^3$ significantly reduced T-SOD and GSH-Px activities and elevated the MDA content in the nasal mucosa (Fig. 3). These results demonstrate that $PM_{2.5}$ induces oxidative stress in the nasal mucosa.

Artificial $PM_{2.5}$-induced inflammation in the nasal mucosa

To examine the impact of artificial $PM_{2.5}$ on inflammatory cytokine production in the nasal mucosa, the levels of IL-1, IL-6 and TNF-α in the serum of the tested rats were analyzed using an ELISA. Fig. 4 shows that the levels of three pro-inflammatory cytokines, Il-1, IL-6 and TNF-α, had significantly increased exposure response to artificial $PM_{2.5}$ at concentrations of 200, 1,000, and 3,000 $\mu g/m^3$ compared to the control. These results indicate that artificial $PM_{2.5}$ induces the expression of inflammatory cytokines.

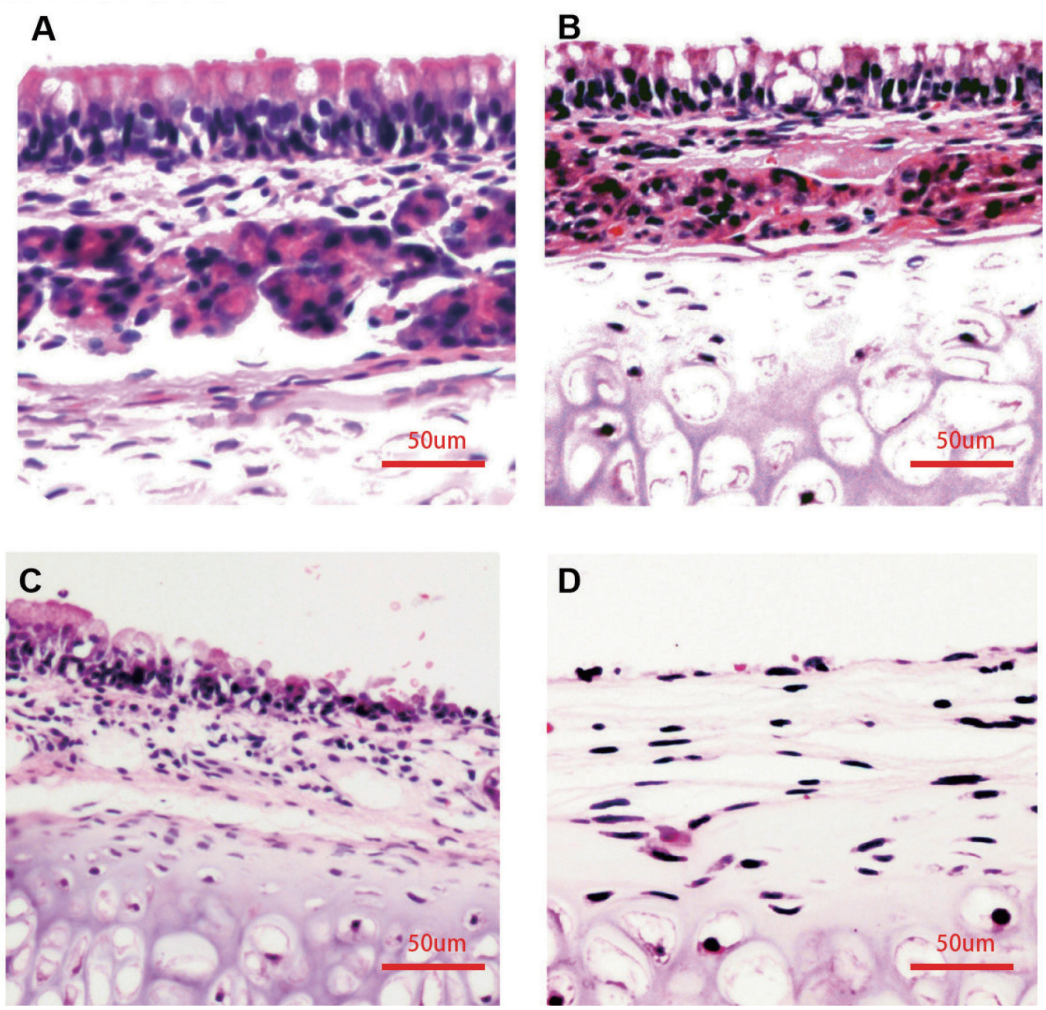

Fig. 2. The histopathological changes of nasal mucosa after artificial $PM_{2.5}$ inhalation in hour groups (hematoxylin and eosin staining).
A. The nasal mucosa of the negative control (NC) group. B. The nasal mucosa of the low concentration of artificial PM2.5 exposure ($LarPM_{2.5}$) group. C. The nasal mucosa of the moderate concentration of artificial PM2.5 exposure ($MarPM_{2.5}$) group. D. The nasal mucosa of the high concentration of artificial PM2.5 exposure ($HarPM_{2.5}$) group.

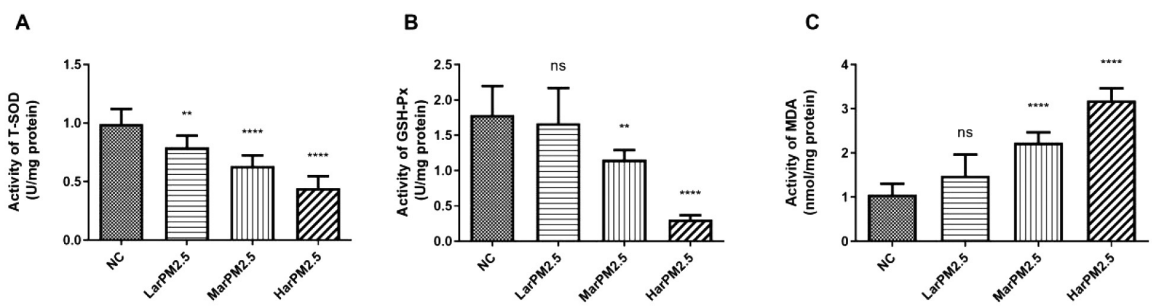

Fig. 3. Artificial $PM_{2.5}$-induced oxidative stress in the nasal mucosa.
Artificial $PM_{2.5}$ reduced total superoxide dismutase (T-SOD) (A) and glutathione peroxidase (GSH-Px) (B) activities, and elevated the malondialdehyde (MDA) (C) content in the nasal mucosa.
NC, negative control group; $LarPM_{2.5}$, a low concentration of artificial $PM_{2.5}$ exposure group, exposed to 200 $\mu g/m^3$ artificial $PM_{2.5}$; $MarPM_{2.5}$, a moderate concentration of artificial $PM_{2.5}$ exposure group, exposed to 1,000 $\mu g/m^3$ artificial $PM_{2.5}$; $HarPM_{2.5}$, a high concentration of artificial $PM_{2.5}$ exposure group, exposed to 3,000 $\mu g/m^3$ artificial $PM_{2.5}$, ns, not significant. $^{**}P < 0.01$, $^{****}P < 0.0001$ vs. control (NC).

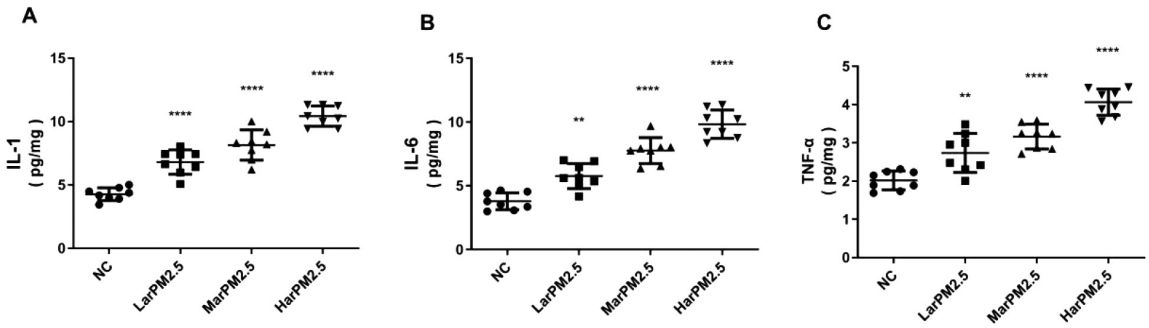

Fig. 4. Artificial $PM_{2.5}$ increases inflammatory cytokines in the nasal mucosa.
The levels of three pro-inflammatory cytokines, interleukin-1 (IL-1) (A), IL-6 (B) and tumor necrosis factor-α (TNF-α) (C), had significantly increased exposure response to artificial $PM_{2.5}$.
NC, negative control group; $LarPM_{2.5}$, a low concentration of artificial $PM_{2.5}$ exposure group, exposed to 200 $\mu g/m^3$ artificial $PM_{2.5}$; $MarPM_{2.5}$, a moderate concentration of artificial $PM_{2.5}$ exposure group, exposed to 1,000 $\mu g/m^3$ artificial $PM_{2.5}$; $HarPM_{2.5}$, a high concentration of artificial $PM_{2.5}$ exposure group, exposed to 3,000 $\mu g/m^3$ artificial $PM_{2.5}$. $^{**}P < 0.01$, $^{****}P < 0.0001$ vs. control (NC).

Discussion

Actual $PM_{2.5}$ in the atmosphere has been linked to a range of adverse health effects (Zhang et al. 2016). In this study, we constructed artificial $PM_{2.5}$ particles that closely matched the composition ratio, size, and morphology of actual $PM_{2.5}$. Furthermore, our in vivo study showed that artificial $PM_{2.5}$ exposure reduces T-SOD and GSH-Px activities, elevates the MDA content in the nasal mucosa, and induces increased levels of pro-inflammatory mediators, including IL-1, IL-6 and TNF-α. Our data showed that artificial $PM_{2.5}$ induces oxidative stress and inflammatory response in vivo. The result is consistent with a previous study showing that exposure to $PM_{2.5}$ has the potential to induce oxidative damage and the inflammatory response in the nasal mucosa of rats (Guo et al. 2017). Our findings suggest that oxidative stress and inflammatory response may play an important role in artificial $PM_{2.5}$-induced nasal lesions and nasal epithelial barrier dysfunction following exposure with artificial $PM_{2.5}$.

A better understanding of the mechanisms of actual $PM_{2.5}$-induced nasal lesions is necessary. However, because of variations in actual $PM_{2.5}$ physical and chemical properties, studying actual $PM_{2.5}$-induced damage to human health has been difficult. Actual $PM_{2.5}$ contains organic and inorganic components, which are involved in its toxicological effects (Feng et al. 2016). Previous studies showed that the primary composition of these particles, metallic elements, induce inflammatory response. Trace metals were the most toxic substances in ambient PM (Ribeiro et al. 2016). Due to the lack of a constant actual $PM_{2.5}$, these studies have lacked repeatability. In another of our studies, actual $PM_{2.5}$ was collected on Whatman 41 filters using TSP/PM_{10}/$PM_{2.5}$-2 samplers. The sample filter containing $PM_{2.5}$ was immersed in deionized water and sonicated using a KQ-50B water-bath sonicator. The obtained actual $PM_{2.5}$ was used in cell experiments (Hong et al. 2016). In the present study, actual $PM_{2.5}$ was collected every day for four months (January, April, July and October) of 2013 in Shanghai, China. From these samples, eleven metals and six inorganic ions were detected and analyzed. We measured the main toxic metals and ions in actual $PM_{2.5}$, and then in accordance with the metal and ion constituents of actual $PM_{2.5}$, we constructed artificial $PM_{2.5}$ particles with a stable composition to maintain consistency for further study. All artificial $PM_{2.5}$ components were fully ground, and scanning electron microscopy revealed that artificial $PM_{2.5}$ was roughly the same size and shape as actual $PM_{2.5}$. The compositions and contents in artificial $PM_{2.5}$ and actual $PM_{2.5}$ we had measured were almost the same. Our findings suggest that artificial $PM_{2.5}$ used in this research could be suitable for experimental study because its physical and chemical properties are relatively constant. This is of great significance for maintaining study repeatability in $PM_{2.5}$ toxicology research. Considering that nasal drips in traditional experiments cannot simulate the real situation of actual $PM_{2.5}$ inhaled by rats in the air. We had tried to change artificial $PM_{2.5}$ into solid aerosol directly to simulate the real state of actual $PM_{2.5}$ in air pollution in the pre-experiment, but the effective concentration of artificial $PM_{2.5}$ in the exposure chamber could not meet the experimental requirements, and the concentration could not be maintained for an enough time. The $PM_{2.5}$ concentration in the $PM_{2.5}$ inhalation exposure system was controllable and measurable, and could be maintained stable for a long time. Thus, we choose to use the liquid aerosol generator (hrh-wag6, Beijing, China) to generate liquid aerosols in the $PM_{2.5}$ inhalation exposure system.

The $PM_{2.5}$ inhalation exposure system used in this study was previously described. The suction gas per minute of a 300 g rat was approximately 200 mL (Zhao et al. 2012). Inhalation exposure to 200, 1,000, and 3,000 $\mu g/m^3$ of artificial $PM_{2.5}$ for 3 h/day over 30 consecutive days delivered total amounts of 0.22 mg, 1.08 mg, and 3.24 mg of $PM_{2.5}$, respectively, in this study. The total $PM_{2.5}$ exposure levels in this study therefore corresponded to previous

intratracheal instillation studies, which defined a low dose as 0.2 mg/rat, a medium dose as 0.8 mg/rat, and a high dose as 3.2 mg/rat (Wang et al. 2015). The levels of $PM_{2.5}$ exposure in our study were 8-fold ($LarPM_{2.5}$), 40-fold ($MarPM_{2.5}$), and 120-fold ($HarPM_{2.5}$) higher than the air quality guidance issued by the World Health Organization (WHO) ($PM_{2.5}$, 25 $\mu g/m^3$ over a 24 h period).

In vitro studies have found that oxidative stress and the inflammatory response play an important role in the nasal epithelium, contribute to the impairment of nasal epithelial barrier following $PM_{2.5}$ exposure, and further decrease cell viability (Hong et al. 2016). However, its underlying molecular mechanism has remained largely unknown, especially because of a lack of toxicological studies in vivo. In the present study, our data indicated that artificial $PM_{2.5}$ triggers a decrease in activities of SOD and GSH-Px and elevates MDA levels and inflammatory cytokines in rat nasal mucosa exposed to $PM_{2.5}$. These results support the viewpoint that ROS may be a consequence of the inflammatory response in nasal injury and that the inflammatory response induced by $PM_{2.5}$ exposure is related to nasal injury. This study revealed that $PM_{2.5}$ increases the expression of IL-1, IL-6 and TNF-α. These findings suggest that $PM_{2.5}$ may initiate and augment local inflammation and lead to disease exacerbation by stimulating the production of IL-1, IL-6 and TNF-α. Our study fills the gap of in vivo toxicological studies in this field.

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Conflict of Interest

The authors declare no conflict of interest.

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