Alterations of IL-1beta and TNF-alpha expression in RAW264.7 cell damage induced by two samples of PM$_{2.5}$ with different compositions

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Abstract
Fine particulate matter 2.5 (PM$_{2.5}$) has been demonstrated by previous studies to be associated with cell damage. To explore the impact of the composition of PM$_{2.5}$ on PM$_{2.5}$-mediated inflammation, this study investigated the composition of PM$_{2.5}$ collected during the wintertime indoor heating season and observed its inflammatory effect. Samples were collected during the heating season from December 5, 2017, to January 8, 2018, in Xi’an. Compositions of organic carbon (OC), elemental carbon (EC), and water-soluble ions were analysed. Two representative samples

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(sample 1 and 2) were selected with significant differences in compositions. They were configured into four concentrations (0.1 μg/mL, 1 μg/mL, 10 μg/mL, 20 μg/mL) and used as interventions on RAW264.7 cells for 4 h and 24 h separately. Cell viability was detected by CCK-8. Tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) gene and protein expression levels were detected by real-time quantitative real-time polymerase chain reaction (RT-qPCR) and western blotting. The results showed that the cell viability of sample 1 intervened cells at 4 h and 24 h was lower than that of sample 2. IL-1β gene in most PM2.5 intervention groups was lower than in the control group. Protein expression was higher at 4 h than at 24 h. In conclusion, PM2.5 components influence cell viability and expression of IL-1β and TNF-α, while high concentrations of NO3−, Cl−, Na+, K+, Mg2+, Ca2+; and others in the PM2.5 composition have a significant harmful effect.

**Keywords**
IL-1β, TNF-α, macrophage, PM2.5

**Introduction**

With rapid urbanization and economic growth, China has undergone a series of serious challenges in dealing with air pollutants. Among many pollutants, fine particulate matter 2.5 (PM2.5) gets a lot of attention because of its special composition and the uncertainty related to its harmful effects on health. A great number of epidemiological studies have found that respiratory, cancer and cardiovascular mortality are closely associated with PM2.5 exposure.1,2 Research has demonstrated that the adverse health effects of PM2.5 are associated with its physicochemical properties, such as size, surface area, and chemical composition.3 The mechanisms associated with the harmful effects of PM2.5 have not yet been fully understood, but there is some evidence that inflammatory processes may play a key role in the induced pathophysiology.4

It is well known that PM2.5 is a complex mixture of solid and liquid airborne particles, and different PM2.5 components have different effects on cell inflammatory responses.5 In China, Xi’an is one of the most heavily polluted cities, and as a result, it has experienced poor air quality in recent years.6 Several studies in Xi’an have examined the negative effects of ambient air pollutants on various outcomes, including mortality and respiratory diseases.7,8 But few studies have focused on the specific hazards of certain PM2.5 components, and their influence on inflammatory processes and oxidative stress. Such study is necessary, however, in order to contribute to a better understanding of the inflammatory processes caused by the particulate matter of varying composition.

Macrophages play an important role in the immune system of the human body,9 and are a key part of the body’s response to PM2.5. Typically, pathogens that enter the body are removed by macrophages through the process of phagocytosis. Macrophages also increase the viability of other immune-functioning cells, prompting other lymphocytes to join in the fight against the invading pathogens.10 Once PM2.5 enters the respiratory tract, as with other pathogens, macrophages will phagocytose it and migrate to the end of the bronchioles. These macrophages will then be removed by the mucus cilia clearance system. Increasing PM2.5 concentration in the lungs can cause damage to macrophages, hindering phagocytosis, and causing the release of a variety of inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). Through this
process, PM$_{2.5}$ can cause lung damage, enter the systemic circulation, and induce the release of inflammatory cytokines in multiple locations of the body.$^{11}$

In this study, the composition of PM$_{2.5}$ and the inflammatory response induced in macrophages were investigated. We used a high-flow sampler and quartz fibre membrane to collect PM$_{2.5}$ samples from the same location for 35 days of the wintertime indoor heating season in Xi’an. Through analysis of the main components of collected samples, two samples (sample 1 and sample 2) with significant composition differences were selected as the interventions for murine macrophages (RAW264.7). The viability of RAW264.7 and IL-1β, TNF-α expression were then investigated. After that, the differences between the PM$_{2.5}$ components and treatment results of the two samples were compared. This study aims to help in the effort to effectively reduce the risk of particulate matter by providing information that allows for more targeted preventive measures.

**Materials and methods**

**Sample collection and preparation**

The study selected Xi’an China as the sampling point. PM$_{2.5}$ samples were selected from samples that were continuously collected from December 5, 2017, to January 8, 2018, for over 35 days. The sampling site was located on the roof of the second floor of the main building of the Institute of Earth Environment, at the Chinese Academy of Sciences (34.22°N, 108.88°E). This location is situated at the intersection of the high-tech industrial development zone, high-tech residential area, and business district. There is no obvious carbon aerosol pollution emission source around, and the terrain is open, with relatively few tall buildings. The sampling head of the aerosol instrument was 10 m above the ground. There was no barrier higher than the height of the instrument around the observation field, and the atmospheric convection was smooth.

A high-flow sampler (TISCH, TE6070DV-BL, 1.13 m$^3$/min) was used to collect PM$_{2.5}$ samples for the whole day during the winter heating season (from December 5, 2017, to January 8, 2018) over the 35-day collection period. The sampling membrane was a quartz fibre membrane (10 × 8 inch, Whatman QM/A). Quartz fibre membranes were pre-baked (450 °C for 6 h) and then kept at constant temperature (20 °C ± 5 °C) and humidity (40% ± 2%) for 48 h. At the same time, a Teflon membrane (R2PJ047, Pall Life Sciences) with a diameter of 47 mm using a mini vol (5 L/min) sampler was used for subsequent analysis of metal elements. The samples were stored in a freezer (−18°C) before analysis. For the cell experiment, the collected PM$_{2.5}$ samples were dried at high temperatures, and the flocculent was collected and sterilized with liquid nitrogen for one day. The solution was dissolved in a cell medium containing 10% fetal bovine serum (FBS), and the concentration was configured to 2 mg/mL for preservation at −80°C. The particulate solution was re-mixed before each use.

**Sample analysis**

**Mass concentration of PM$_{2.5}$**

The mass concentration of PM$_{2.5}$ was analysed by gravimetric analysis. This value was obtained by dividing the mass difference between the filter membrane before and after
sampling by the sampling volume. Before weighing, the blank filter membrane and the sample filter membrane were placed in a dryer for more than 72 h. The scale of one part per million (Mettler M3, Switzerland) was used for weighing, and the error of the two weighing results was less than 5 g. All samples deducted the weight of the blank.

**Organic carbon (OC), elemental carbon (EC) of PM$_{2.5}$**

Organic carbon (OC) and element carbon (EC) in PM$_{2.5}$ were analysed using the DRI Model 2001 thermo-optic carbon analyser developed by the Desert research institute.\textsuperscript{12,13}

**Water-soluble ions of PM$_{2.5}$**

Water-soluble ions in PM$_{2.5}$ were determined by Dionex-600 ion chromatography. The ions included nitrate ($\text{NO}_3^-$), sulfate ($\text{SO}_4^{2-}$), ammonium ($\text{NH}_4^+$), and other ions ($\text{Na}^+$, $\text{K}^+$, $\text{Mg}^{2+}$, $\text{Ca}^{2+}$, $\text{Cl}^-$). Firstly, a certain amount of quartz membrane filter was put in a centrifuge tube, and 30 mL ultrapure water ($R > 18.2 \, \text{M} \, \Omega$) was then added. After 1 h of ultrasound, the solution was put into the decolorizing shaker and vibrated for 1 h. Then the solution was filtered into a clean centrifuge tube using a 0.45 m drainage filter head (MEMBRANA) and stored in a 4°C refrigerator for later measurement. The anion detection limit was less than 0.027 $\mu$g/mL.

**Cell experiment**

**RAW264.7 cell cultivation and grouping**

The RAW264.7 cells were from the American-type culture collection (ATCC). The cell culture medium consisted of Dulbecco’s modified Eagle’s medium/Nutrient Mixture F-12 (DMEM/F12, HyClone, Thermo Scientific, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), and 1% penicillin-streptomycin solution (HyClone, Thermo Scientific, Logan). Cells were cultured at 37 °C in a humidified incubator containing 5% CO$_2$. Cells were cultured and grouped by different PM$_{2.5}$ blending solutions with varying treatment concentrations (0.1 $\mu$g/mL, 1 $\mu$g/mL, 10 $\mu$g/mL, 20 $\mu$g/mL). According to the pre-experiment trials and data from past experiments,\textsuperscript{14–16} two different time points (4 h, 24 h) were used for the intervention. Two PM$_{2.5}$ samples with significant differences in compositions (sample 1 and sample 2) were selected for the treatment.

**Cell viability**

CCK-8 Kit (Boster AR1160-500) was used to detect cell proliferation and cytotoxicity. After cell passage, 100 $\mu$L of cell suspension was inoculated into a 96-well plate at a density of $2 \times 10^4$ cells per well before treatment. The cells were incubated at a constant temperature for 24 h. The mother liquor of PM$_{2.5}$ was diluted to 0.1 $\mu$g/mL, 1 $\mu$g/mL, 10 $\mu$g/mL, and 20 $\mu$g/mL, with five parallel wells for each concentration. After 24 h of culturing, 10 $\mu$L of the PM$_{2.5}$ was added to each well according to the designed concentration gradient. Cells were incubated in two groups, one for 4 h and the other for 24
10 μL CCK-8 solution was then added to each well, and the cells were incubated for 65 min. Absorbance was measured at 450 nm. Each group was repeated 4 times.

Reverse transcription-quantitative real-time polymerase chain reaction (RT-qPCR)

The total RNA of RAW264.7 macrophages was isolated using RNA fast200 kit (Feijie biology). The quality and concentration of the collected RNA was assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA). First-strand cDNA was then synthesized using a cDNA reverse transcription kit (Thermo Fisher Scientific K1651). The forward and reverse primer (Aoko Dingsheng Co. Ltd, Beijing, China) were as follows: TNF-α (5′-CAGGCGGTGCCTATGTCTC-3′, 5′-CGATCACCCCCGAAGTT CAGTAG-3′); IL-1β (5′-TTCAGGCAGGCAGTATCCTC-3′, 5′-GAAGGTCCAC GGGAAAGACAC-3′); GAPDH (5′-GAAGGTGGTGAAGCAGGCATCT-3′, 5′-CGGCATCGAAGGTGGAAGGTG-3′). GAPDH was used as an internal control. The reverse transcription products were used for repeated real-time quantitative real-time polymerase chain reaction (RT-qPCR) analysis performed with an Mx3005P qPCR System (Agilent Technologies, Inc.). The cycling settings of the RT-qPCR process were as follows: 15 min at 95°C, followed by 40 cycles (95°C for 10 s, 60°C for 20 s, and 72°C for 20 s). The gene expression was assessed using the 2−ΔΔCt method. The experiment was repeated three times for both time points (4 h and 24 h).

Western blotting analysis

Proteins of TNF-α and IL-1β were extracted by a RIPA lysis buffer (Xi ‘an Guoan Biotechnology LTD). The quantity of the total protein extracted was quantified using the BCA protein quantitative Kit (PA115, TianGen Biotech, Beijing) and NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA). For equivalent loading doses per lane, 15 μg of protein from each group was used. The proteins were separated by SDS-PAGE (Boster AR1112). After electrophoresis, the proteins were transferred to the PVDF membrane (Hybond ECL, Amersham). Then, the membrane was incubated at room temperature (RT) in a blocking solution (TBST, Tris-buffered saline (TBS, PH 7.4) with 5% skimmed milk powder and 0.1% Tween 20) for 2 h. Later, the membrane was incubated with TNF-α (1: 1500, Bioss), IL-1β (1:1500, Bioss), and GAPDH (1: 10000, Bioss) antibodies separately at 4°C overnight. GAPDH was used as an internal control. The secondary antibody was the horseradish peroxidase-conjugated (HRP-conjugated) immunoglobulin G (IgG) antibody. It was diluted by blocking solution to 1:10000. For visualizing the immunoreactive protein, an enhanced chemiluminescence detection kit (ECL, Thermo Scientific, USA) was used. The signals were analysed via Image J Software (NIH Image, USA). The ratio of the protein expression levels of the TNF-α and IL-1β (normalized to GAPDH) for 4 h and 24 h were then calculated.

Statistical analysis

The software SPSS 23 was used for data analysis, and one-way analysis of variance (one-way ANOVA) and t-test were used in the analysis. One-way ANOVA was used to compare the
significance between treatment groups and control groups. A T-test was used to compare the differences between the two groups at the same concentration. If $P<0.05$, the data was statistically significant. Three biological replicates were performed in this experiment.

**Results**

**Composition of PM$_{2.5}$**

PM$_{2.5}$ mass concentrations of sample 1 and sample 2 were 360.03 $\mu$g/m$^3$ and 71.71 $\mu$g/m$^3$ respectively. The concentrations of various components including OC, EC, and watersoluble ions were analysed, and the concentration of organic matter (OM) was estimated at 1.6 times OC concentration$^{17}$ (Figure 1A). For sample 1, the top five components were others (undetected part), OM, NO$_3^-$, other ions (Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Cl$^-$), and SO$_4^{2-}$ in order of concentration percentage from largest to smallest. For sample 2, the top five components were others, OM, SO$_4^{2-}$, NO$_3^-$ and NH$_4^+$, also in descending order. Others accounted for the largest proportion in both samples (sample 1: 47.68%, sample 2: 40.15%). Water-soluble ions were 31.43% and 35.36% of PM$_{2.5}$ sample 1 and sample 2 compositions respectively. Of the components with significant differences in proportion between the two samples, SO$_4^{2-}$ made up 14.85% of sample 2 PM$_{2.5}$ concentrations, which was about 2.4 times the SO$_4^{2-}$ proportion of sample 1 (6.27%). NO$_3^-$, other ions (Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Cl$^-$) and others of sample 1 were higher than sample 2 in concentration proportion. Cl$^-$, for example, had a concentration in sample 1 (2.63%) that was about 2.8 times higher than that of sample 2 (0.93%) (Figure 1B, Figure 1C).

**Results of cell viability**

RAW264.7 cells were treated with two PM$_{2.5}$ samples of four different concentrations. The cell survival rate of sample 1 at 4 h and 24 h showed no significant difference from that of the control group (Figure 2A, Figure 2B). For sample 2 at 24 h, the survival rate was higher than that of the control group. The differences were significant when the concentrations were 1 $\mu$g/mL and 10 $\mu$g/ml (1 $\mu$g/mL $P=0.034$, 10 $\mu$g/mL $P=0.003$) (Figure 2B). In Figures 2(a) and 2(b), almost all cell viability of sample 1 at both time points was lower than that of sample 2. The results were both significant when the concentration was 1 $\mu$g/mL (4 h: sample 1 versus sample 2 $P<0.001$; 24 h: sample 1 versus sample 2 $P=0.009$). In Figures 2(c) and 2(d), for the same sample, the cell survival rates were different for the two time points, with the 4 h cell survival rates being lower than the 24 h rates (Figure 2C, Figure 2D). Comparing both samples, the cell survival rates were significantly different when the concentration was 0.1 $\mu$g/mL and 1 $\mu$g/mL (sample 1: 4 h versus 24 h, 0.1 $\mu$g/mL $P=0.001$, 1 $\mu$g/mL $P<0.001$; sample 2: 4 h versus 24 h 0.1 $\mu$g/mL $P=0.041$, 1 $\mu$g/mL $P=0.007$).

**Results of gene expression**

***IL-1$\beta$ mRNA expression levels after PM$_{2.5}$ intervention***

For sample 1, IL-1$\beta$ gene levels were significantly lower than that of the control group at both 4 and 24 h for every intervention concentration (Figure 3A, Figure 3B). The results
of IL-1β gene levels at 4 h were significantly higher in sample 2 than in sample 1 when the intervention concentration was 0.1 μg/mL ($P<0.001$) and 20 μg/mL ($P=0.001$). When detected at 24 h, IL-1β gene levels were significantly higher in sample 2 than in sample 1 when intervention concentrations were also 0.1 μg/mL ($P=0.005$) and 20 μg/mL ($P=0.003$). On the other hand, IL-1β gene levels were higher in sample 1 than in sample 2 when intervened at 1 μg/mL ($P=0.026$) and 10 μg/mL ($P<0.001$) for 24 h (Figure 3A, Figure 3B). IL-1β gene levels were significantly higher at 24 h than at 4 h under the sample 1 intervention at 0.1 μg/mL, 1 μg/mL, and 10 μg/mL concentrations (Figure 3C). IL-1β gene levels at 4 h were higher than at 24 h when intervened by sample 2 (Figure 3D).

**TNF-α mRNA expression levels after PM$_{2.5}$ intervention**

TNF-α gene levels were significantly lower than that of the control group for sample 2 when PM$_{2.5}$ concentrations were 0.1 μg/mL and 1 μg/mL, and significantly higher than the control group when concentrations were 1 μg/mL, 10 μg/mL, and 20 μg/mL. When detected after 4 h, TNF-α gene levels of sample 2 were higher than that of sample 1 and the results were significant when the intervention concentration was 10 μg/mL.
When detected at 24 h, TNF-α gene levels of most sample 2 groups were higher than that of sample 1 and the results were significant when concentrations were 0.1 μg/mL (P = 0.032) and 10 μg/mL (P = 0.024) (Figure 4A, Figure 4B). The results of the TNF-α gene expression levels at 24 h were higher than at the 4 h time point (Figure 4C, Figure 4D).

Western blotting results of IL-1β and TNF-α

**IL-1β protein expression after PM$_{2.5}$ intervention**

Western Blotting results show the protein expression level of IL-1β and TNF-α (Figure 5A). For IL-1β protein expression, both sample 1 and sample 2 results at 4 h were higher than the control group whereas at 24 h, the results were lower than the control group (Figure 5B, Figure 5C). Sample 1 intervention group protein expression levels at 24 h were significantly higher than sample 2 when intervention concentrations were 0.1 μg/mL (P < 0.001), 1 μg/mL (P = 0.014) and 20 μg/mL (P = 0.034) (Figure 5C).
For both sample 1 and sample 2, IL-1β protein levels were higher at 4 h than at 24 h at every intervention concentration. For sample 1, the results were significantly higher at 4 h than at the 24 h timepoint when intervention concentrations were 1 μg/mL (P = 0.021), 10 μg/mL (P < 0.001), and 20 μg/mL (P = 0.016) (Figure 5D). For sample 2, intervention results were significantly higher at 4 h than at 24 h when concentrations were 0.1 μg/mL (P < 0.001), 1 μg/mL (P = 0.012), 10 μg/mL (P = 0.049) and 20 μg/mL (P < 0.001) (Figure 5E).

**TNF-α protein expression after PM$_{2.5}$ intervention**

TNF-α protein expression levels of both samples at 4 h and 24 h were all higher than the control group (Figure 5F, Figure 5G). When detected at 4 h, the results of the sample 2 intervention group were higher than the sample 1 intervention group when the intervention concentrations were 0.1 μg/mL, 1 μg/mL, and 10 μg/mL. The results were significant at 0.1 μg/mL (P < 0.001) and 1 μg/mL (P = 0.030), (Figure 5F). For the 24 h group, sample 2 results were higher than the sample 1 intervention group results when intervention concentrations were 10 μg/mL and 20 μg/mL (Figure 5G). Both sample intervention
groups were higher at 4 h than at 24 h when concentrations were 1 μg/mL, 10 μg/mL, and 20 μg/mL. For sample 1, the results were significant when intervention concentrations were 1 μg/mL ($P = 0.017$), 10 μg/mL ($P = 0.032$), and 20 μg/mL ($P = 0.013$) (Figure 5H). For sample 2, the results were significant when intervention concentrations were 0.1 μg/mL ($P < 0.001$), 1 μg/mL ($P = 0.018$), and 20 μg/mL ($P = 0.026$) (Figure 5I).

**Discussion**

In recent years, there have been many studies covering the harmful effects of PM$_{2.5}$

Due to the complex composition of PM$_{2.5}$ and its significant variation with time, space and emission sources, the specific components’ impact on health has been difficult to determine. This study was performed on RAW264.7 under the intervention of PM$_{2.5}$ samples of different components in varying proportions at different time points and concentrations. We compared intervention effects such as survival rate, transcription, and translation of cell inflammatory cytokines. From this study, it can be seen that the specific composition of PM$_{2.5}$ can affect the degree of inflammatory response. Samples with higher concentrations of NO$_3^-$, Cl$^-$, Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, and others may have a greater toxic effect.
Figure 5. (a) protein expression levels at different concentrations and time points of pm$_{2.5}$ samples were detected by western blotting. Cells were exposed to two PM$_{2.5}$ samples at four concentrations (0.1, 1, 10, 20 μg/mL) for 4 h and 24 h. GAPDH was used as the internal control. (B) Sample 1 and sample 2 IL-$\beta$ relative protein expression levels after intervention at 4 h. (C) Sample 1 and sample 2 IL-$\beta$ relative protein expression levels after 24 h of intervention. (D) Sample 1 IL-$\beta$ relative protein expression at 4 h and 24 h. (E) Sample 2 IL-$\beta$ relative protein expression at 4 h and 24 h. (F) Sample 1 and sample 2 TNF-$\alpha$ relative protein expression levels after 4 h of intervention. (G) Sample 1 and sample 2 TNF-$\alpha$ relative protein expression levels after 24 h of intervention. (H) Sample 1 TNF-$\alpha$ relative protein expression for 4 h and 24 h timepoints. (I) Sample 2 TNF-$\alpha$ relative protein expression for 4 h and 24 h timepoints. $^\# P<0.05$ versus the control group. $^{##} P<0.01$ versus the control group. $^{*} P<0.05$ versus same concentration of PM$_{2.5}$ sample. $^{**} P<0.01$ versus the same concentration of PM$_{2.5}$ sample.
Previous studies mostly discuss the relationship between PM$_{2.5}$ concentration and cell viability, and the results of this study show that the higher the PM$_{2.5}$ mass concentration, the lower the cell survival rate,\textsuperscript{21} this indicates a dose-response relationship\textsuperscript{22} where the toxic effect of PM$_{2.5}$ on macrophages increases with the increase in PM$_{2.5}$ concentration, leading to apoptosis of more cells and reduced cell survival rate.\textsuperscript{23} However, few studies have discussed Xi’an PM$_{2.5}$ components and cell viability.

In this study, we focused on the composition of PM$_{2.5}$ in Xi’an. For the two samples (sample 1 and sample 2), the cell viability of sample 2 intervened cells was higher than in sample 1 at the same time point and concentration. We also compared the differences between the two samples’ components. For the component that made up the largest proportion, “others” (composed of crustal metals, trace heavy metals, and some unknown components), the difference between the two samples was relatively small. The second-largest components, OM and EC, came mainly from combustion sources, with EC, in particular, typically released during incomplete combustion in primary emission OM was calculated from OC by considering the unmeasured elements (H, O, N, and S) in the organic compounds. The proportions of OM and EC in sample 2 were a little higher than in sample 1. However, the proportions in sample 1 of NO$_3^−$, Cl$^−$, Na$^+$, K$^+$, Mg$^{2+}$, and Ca$^{2+}$ were all higher than in sample 2, indicating that components may be influencing factors for the decrease of cell viability observed in the sample 1 intervention group. Previous studies have shown that Cl$^−$ is likely to be associated with coal combustion and secondary aerosol (mixed sources) while some of the NO$_3^−$ might come from motor vehicle emissions\textsuperscript{24} and the rest of the NO$_3^−$ is associated with secondary inorganic aerosol.\textsuperscript{25} Na$^+$, K$^+$, Mg$^{2+}$, and Ca$^{2+}$ have been found to have a relatively strong association with biofuel combustion,\textsuperscript{26} and Mg$^{2+}$ and Ca$^{2+}$ have been used as indicators for construction dust (including fugitive dust and resuspended road dust) in Xi’an.\textsuperscript{27}

Alveolar macrophages are the predominant cells that clean inhaled particulate matter in the lung.\textsuperscript{28} They produce pro-inflammatory mediators implicated in local as well as systemic inflammatory responses, leading to the recruitment of macrophages.\textsuperscript{29,30} The increasing amount of PM$_{2.5}$ will induce an inflammatory response of the macrophages and stimulate the lung epithelial cells and mononuclear macrophages, releasing IL-1β, TNF-α. IL-1β expression can promote the vitality of cells required for various immune responses, increase leukocytosis, cause redness and swelling of the skin and promote the production of interleukins and interferons. TNF-α also plays an important role in promoting cytokine production. When invading exogenous substances stimulate lung cells, TNF-α will be released in large quantities to mediate inflammatory responses. Therefore, IL-1β and TNF-α both play important roles in local and systemic inflammation.\textsuperscript{31} Previous studies on IL-1β and TNF-α have focused on increased expression levels after exposure to PM$_{2.5}$ in addition to other studies, which have focused on PM$_{2.5}$ activating signalling pathways to increase the release of IL-1β.\textsuperscript{32} Our study, however, focused on PM$_{2.5}$ composition and treatment time’s effect on cell viability and IL-1β, TNF-α expression.

The results of IL-1β gene expression showed that at 24 h, differences between sample 1 and sample 2 in the four concentration groups were statistically significant. When intervened at both 1 μg/mL and 10 μg/mL concentrations, IL-1β gene levels in sample 1 were higher than those in sample 2 at 24 h. Results of IL-1β western blotting showed that at the
24 h time point when intervened at 0.1 μg/mL, 1 μg/mL, 20 μg/mL concentrations, IL-1β protein expression levels in sample 1 (which had higher proportions of NO₃⁻, Cl⁻, Na⁺, K⁺, Mg²⁺, and Ca²⁺) were significantly higher than in sample 2. The above results suggest that the differences in PM₂.₅ components may have affected IL-1β transcription and translation. The results of TNF-α showed that the RNA expressions of the sample 1 groups were all greater than that of sample 2 at 4 h, and the RNA expressions of sample 2 were mostly greater than that of sample 1 at 24 h except for the group with the highest concentration (20 μg/mL). This phenomenon suggests that PM₂.₅ components may affect the transcription rate of TNF-α. Results of TNF-α western blotting showed at 4 h, when intervened at 0.1 μg/mL, 1 μg/mL TNF-α protein expression levels in sample 2 (which had higher proportions of OM and EC) were significantly higher than in sample 1. For the majority of the RAW264.7 cells in both sample 1 and sample 2 treatment groups, the transcription and expressions level of IL-1β were lower than that in the control group, which may be related to the autophagy caused by PM₂.₅. The transcription level of TNF-α for the 24 h time point, and the expression level for 4 h and 24 h were higher than the control group. This suggests that PM₂.₅ caused cell inflammatory damage.

There are some limitations of this study. First of all, due to limited conditions, this study only used Western Blot to explore the expression of IL-1β and TNF-α and did not conduct an enzyme-linked immunosorbent assay (ELISA) analysis on the components secreted by cells. Because of its different focus, the experiment also lacked LPS to test for endotoxin. Secondly, the decrease in IL-1β may also be due to reduced cell viability. Thirdly, the “others” component in the PM₂.₅ samples needs to be further analysed to ascertain the composition and how the different constituents can affect health.

This study found that PM₂.₅ with higher concentrations of NO₃⁻, Cl⁻, Na⁺, K⁺, Mg²⁺, Ca²⁺, and others may have a relatively higher harmful influence when compared to PM₂.₅ with lower concentrations of these components. This means that in heavy pollution weather, it is possible to control PM₂.₅ emissions in a more targeted and efficient way. For example, stricter control measures of enterprises’ use of biofuel and coal can be implemented, use of a surfactant to reduce dust can also be introduced. In this way can we achieve the goal of efficiently improving the environmental conditions and promoting public health.

**Conclusion**

PM₂.₅ components influence the cell activity and the transcription and translation of IL-1β and TNF-α, high concentrations of NO₃⁻, Cl⁻, Na⁺, K⁺, Mg²⁺, Ca²⁺, and others in the PM₂.₅ composition have a significant harmful effect.

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**Author contributions**

Conceptualization: Jieting Zhou and Yizhen Lyu; Methodology: Jing Han and Guocheng Hu; Formal analysis and investigation: Jin Li, Liang Wang; Writing - original draft preparation:
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Data availability statement
The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request

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