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RESEARCH ARTICLE

# Investigating the Cytotoxicity and Inflammatory Response of PM2.5 on Human Lung Cells

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ABSTRACT: Particulate Matter 2.5 (PM2.5) pollution is a significant environmental concern, comprising fine inhalable particles with diameters of 2.5 micrometers or smaller. When inhaled, these microscopic particles pose serious health risks to humans, leading to various adverse health effects. PM2.5 particles primarily originate from multiple sources, such as combustion processes, industrial activities, and transboundary pollution. This study investigates the impact of PM2.5 on human lung cells and its potential effects on inflammation and cell viability. MRC5 human lung cells were subjected to different concentrations of PM2.5, ranging from 0% to 5%, and incubated for 48 hours. Cell viability was assessed using fluorescent markers, while cell morphology and cell death were observed in 3D cell culture models. Additionally, gene expression levels of inflammation markers TNF-α, IL-8, IL-6, and IL-2 were analyzed through gel electrophoresis and quantified. The results of this study support the hypothesis that increased concentrations of PM2.5 negatively impact human lung cell viability. A concentration-dependent decrease in cell viability was observed, with higher PM2.5 levels significantly reducing live cells. Also, PM2.5 exposure resulted in alterations in cell morphology, with treated cells appearing scattered and disorganized compared to control cells. This study also demonstrated a clear association between PM2.5 exposure and increased gene expression levels of inflammation markers TNF-α, IL-8, and IL-6. These genes exhibited higher band intensities in the 5.0% PM2.5 treatment group, suggesting a link between PM2.5 exposure and heightened inflammatory responses in human lung cells. In conclusion, this research underscores the adverse effects of PM2.5 pollution on human lung cells, emphasizing the importance of mitigating PM2.5 emissions and implementing measures to reduce human exposure to this hazardous particulate matter. The findings contribute to our understanding of the health risks associated with PM2.5 pollution. They may inform public health and environmental policies to improve air quality and reduce the prevalence of related respiratory diseases.

KEYWORDS: Cellular and molecular biology; Molecular biology; Particulate Matter 2.5; Lung; Inflammation.

## Introduction

Particulate Matter, or particle pollution, is a mixture of particles of solids or liquids in the air. It contains microscopic droplets that are so small that they can be inhaled and cause serious health risks.[1] Particulate Matter 2.5 (PM2.5) describes fine inhalable particles with generally 2.5 micrometers or smaller diameters. These particles are inhalable into the lungs, which could induce adverse health effects on the human body.[2]

PM2.5 particles from outdoor air can be directly emitted from gasoline, oil, diesel fuel, or wood combustion. They also come from forest fires or power plants, especially the gases diffusing from diesel-fired devices.[3] Once exposed to the air, these particles can remain airborne for long periods and travel hundreds of miles by the wind. As they are carried over extended distances, the particles settle on certain landscapes, such as the ground or water. They then acidify lakes, streams, and soil through chemical composition, ultimately changing organisms' nutritional balance.[4]

As humans breathe in unhealthy levels of PM2.5, this increases health problems such as heart disease, asthma, and chronic obstructive pulmonary disease (COPD).[5] The particles travel deeply into the respiratory tract, reaching the lungs, causing short-term health consequences such as coughing, sneezing, and shortness of breath. PM2.5 is likely to travel into and deposit on the surface of more profound parts of the lung, which triggers inflammatory responses with reduced lung function in humans.[6]

There are seven chemical components of PM2.5 in general: 10.9% of Sulfate, 16.0% of nitrate, 10.6% of ammonium, 4.3% of elemental carbon, 42.8% of organic matter, 4.9% of chloride, 10.5% of soil or minerals. PM2.5 levels from a global context result in an average of 12 μg/m$^3$.[7] In the case of Korea, PM2.5 is composed of 29% organic carbon, 26% nitrate, 15% sulfate, and 14% ammonium chloride. Korea's PM2.5 levels result in 45 μg/m$^3$ due to its influence from domestic sources, transboundary effects, seasonal patterns, and specific pollutants such as industrial activities. The PM2.5 levels are relatively higher in Korea, primarily because of transboundary pollution caused by vehicle emissions and weather from China.[8]

One in five deaths worldwide is due to fine dust from environmental risks, such as fossil fuels. South Korea's premature death rate from fine dust was 30.5%, the fourth highest in the world.[9] Based on the mortality grid distribution, it was found that approximately 4,000 to 5,000 people die annually in the metropolitan area. Besides high mortality rates, it was reported



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that 92 % of residents in Korea are exposed to particulate matter, resulting in domestic air pollution.[10]

Due to the severity of domestic death rates and air pollution, we collected PM 2.5 from Korea to examine its effects on human lung cells. We linked outcomes to two dependent variables – inflammation and cell viability – hypothesizing that the collected particulate matter would adversely influence the human lung cell by elevating inflammation and degrading viability.

## Methods

***Cell culture and maintenance:***

MRC5 human lung cells were purchased from Korea Cell Line Bank. RPMI1640 cell culture media (Gibco) supplemented with 10% FBS and 1% penicillin and streptomycin were used. The cells were maintained healthy in a 10% $CO_2$ incubator at 37 °C.

***Particulate matter 2.5 (PM2.5) collection:***

PM2.5 was collected in South Korea with a specially designed air filter that collects air particles smaller than 2.5 μm. Then, the soluble fraction of the PM2.5 was collected with the Phosphate-Buffered Saline (PBS) buffer solution. The collected PM2.5 for one day was solubilized with 1 mL PBS and represents 100% solution.

***PM2.5 treatment on MRC5:***

MRC5 cells were prepared in the six-well culture plate. The cells were evenly distributed with the 1 x $10^5$ cells per well. The 0% to 5% PM2.5 concentrations were added to the cells and incubated for 48 hours. Then, the cells were harvested using the Tyrpsin-EDTA solution. The cells were stored at -20 °C for the downstream application.

***3D cell culture using MRC5 cell line:***

After MRC5 cells were trypsinized, 1 x $10^4$ cells were added to the cell culture plate cover, and a total of 20 μL solution was added to make the spheroid structure. Then, 0% to 5% of PM2.5 concentrations were added to the cells and incubated for 48 hours, and this incubation period allowed for observing any cellular responses or changes induced by the exposure to PM2.5 concentrations within the 3D cell culture environment.

***Cell imaging:***

The cell image was captured using a Nikon fluorescent inverted microscope. 2D and 3D culture samples were prepared, and an AO/PI staining solution was employed to identify deceased cells, which were visually distinguished by their orange coloration.

## Result and Discussion

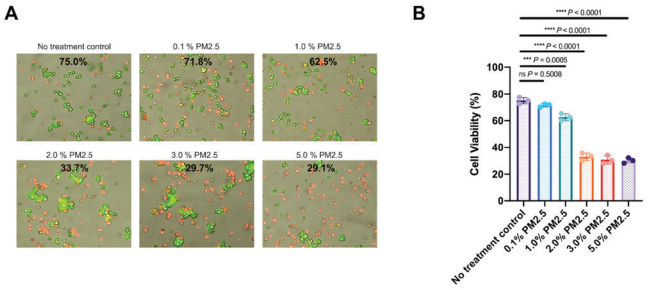


**Figure 1:** PM2.5 decreased the cell viability of normal human lung cell MRC5. (A) Cell image after live (green) and dead (red) cell staining. (B) Bar graph representing the mean and standard deviation of cell viability.

We hypothesized that as the concentration of PM2.5 (%) increases, the human lung cell viability (%) would decrease. The investigation was conducted by creating six different samples of lung cells: A sample with no PM 2.5 treatment, 0.1% of PM2.5 treatment, 1.0% of PM2.5 treatment, 2.0% of PM2.5 treatment, 3.0% of PM2.5 treatment, and 5.0% of PM2.5 treatment. These samples were then incubated for 48 hours. Then, the viability of the cells was analyzed using the cell counting device. The green fluorescent cells demonstrated live cells, while the red fluorescent cells indicated dead cells. Based on the results, it was found that the hypothesis was supported. Figure 1 showed a decreasing trend of live cells as the concentration of PM 2.5 treatment (%) increased. In Figure 1A, the sample with no PM2.5 treatment had the highest number of live cells, indicating cell viability of 75.0%. The sample with 0.1% PM2.5 treatment had 71/8% live cells, and the sample with 1.0% PM2.5 treatment demonstrated 62.5% live cells. When the lung cells had a 1.0% increase in PM2.5 treatment from 2.0% to 3.0%, the concentration of live cells resulted in 33.7% and 29.7%, respectively. In 5.0% of PM2.5 treatment, there were 29.1% live cells. This ultimately shows that with the increase in PM2.5 concentration in human lung cells, there was a corresponding decline in the percentage of viable cells. Based on the outcomes from 2.0% PM2.5 treatment to 5.0% PM2.5 treatment, there was no extreme reduction in the percentage of live cells, which shows that there were small differences between these outcomes. The data ultimately indicates that with the increase in PM2.5 concentration in human lung cells, there was a corresponding decline in the percentage of viable human lung cells.

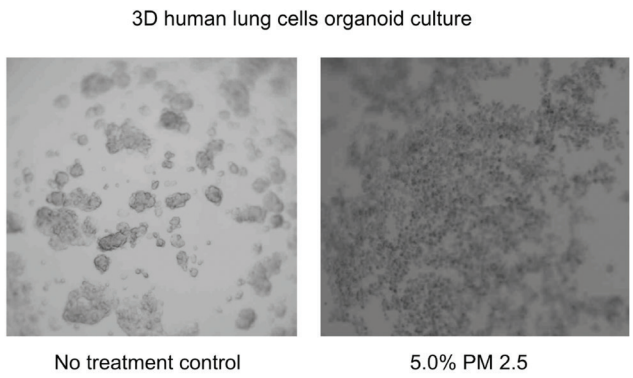


**Figure 2:** PM 2.5 changes the cell morphology of human lung cells MRC5 3D-cultured organoids. It was speculated that cells in the "no treatment control" group may not induce cell death. However, the 5.0% PM 2.5 treated cells exhibited an irregular structure, suggesting cell death, with scattered formations indicative of membrane integrity disruption, a characteristic of the main stage of cell death where the cell membrane bursts.

Our hypothesis posited that an increase in PM2.5 treatment would make the cells more scattered in distinctive directions. Based on the results, it was found that the hypothesis was correct. Figure 2 demonstrated that the human lung cells were showcased in considerable segments with no treatment control, while the cells with 5.0% PM 2.5 treatment were featured in smaller, scattered portions. Although the images were displayed in colorless figures, distinguishing between live and dead cells was unattainable. We speculated that the "no treatment control" cells may not induce cell death. However, as previously noted, the structure of 5.0% PM 2.5 treated cells did not show a particular shape; they exposed themselves in a scattered form, which indicated cell death. Specifically, referring to the main stage of cell death, the cell membrane bursts, which destroys membrane integrity. This triggers the disability of the cell to form small segments yet dispersed portions once they are treated with 5.0% PM 2.5. The results ultimately validated that an augmentation in PM2.5 treatment would lead to greater dispersion of cells in distinct directions, elevating the number of dead cells.

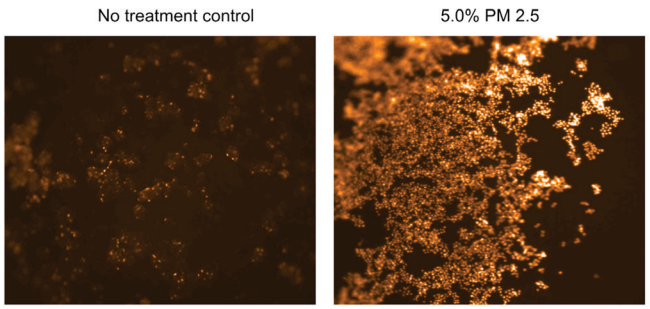


**Figure 3:** Enhanced cell mortality in MRC5 human lung cell 3D organoids with PM2.5 exposure: Utilizing Propidium Iodide (PI) staining, this figure highlights the increase in dead cells (indicated by orange fluorescence) within the 3D cell culture upon PM2.5 treatment.

When using the method for the previous figure, it was challenging to notice the number of dead cells for each 3D culture. Therefore, we stained the cells with the AO/PI staining solutions, which stained the dead cells in orange. Our hypothesis signified that an increase in PM2.5 treatment would elevate the number of cells with orange fluorescent marks – dead cells. The analysis of the obtained results revealed a notable confirmation of the initial hypothesis. Only a limited number of cells exhibited distinct bright-orange markings when examining the human lung cells under the no-treatment control. In stark contrast, the group subjected to a 5.0% concentration of PM2.5 treatment demonstrated that most cells displayed an orange fluorescence, indicating a substantial cellular response to PM2.5 treatment. This discrepancy in fluorescence patterns between the control and treatment groups illustrates the potential impact of PM2.5 exposure on the behavior and characteristics of human lung cells. Referring to the information in the previous results, since the cell membrane integrity is destroyed, the cell forms small segments yet disperses portions, expressing dead cells scattered in distinct directions. This explains that lung cells with 5.0% PM2.5 treatment involved a brighter color scheme of orange fluorescent marks. The outcomes eventually showed that raising the PM2.5 treatment levels would result in a higher occurrence of cells displaying orange fluorescent indicators, indicating cell death (Figure 3).

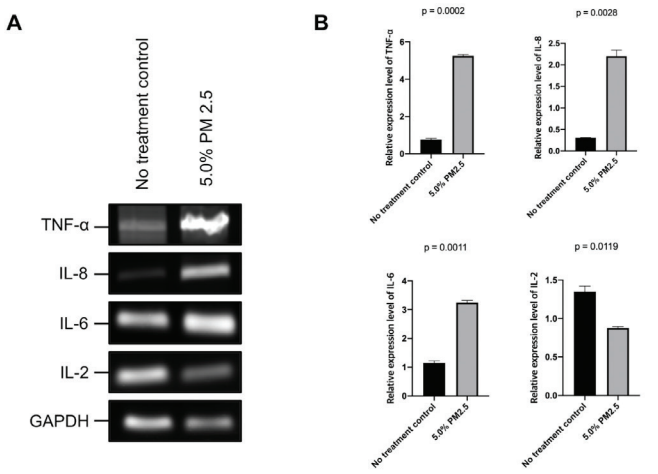


**Figure 4:** PM2.5 increased the inflammation gene expression levels of TNF-α, IL-8, IL-6, and IL-2 in the MRC5 cell line. Our data corroborate the hypothesis that PM2.5 treatment enhances the expression of TNF-α, IL-8, and IL-6, indicating a probable association between PM2.5 exposure and amplified inflammatory responses. (A) The image of gel electrophoresis and the band intensity indicate the expression level of each gene. (B) Quantification of the gene expression level of each gene. GAPDH gene expression level was used for the normalization. An unpaired t-test was used to calculate the p-value.

Our hypothesis mentioned that an elevation in PM2.5 treatment would lead to a heightened expression of inflammation marker genes – TNF-α, IL-8, and IL-6, demonstrating a potential link between PM2.5 exposure and increased inflammatory responses. Based on the results, our hypothesis was supported. The term "expression level" typically refers to the quantity of a specific protein or RNA produced by a gene within a cell or organism. It measures how actively a gene is transcribed and translated into its product. Figure 4A indicated that the relative expression levels of inflammation marker genes, including TNF-α, IL-8, and IL-6, had a band intensity of each gene higher in 5.0% of PM2.5 treatment than that of no treatment control. While these genes showed a higher band


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intensity in the 5.0% PM2.5 treated group, IL-2 genes had a higher band intensity in no treatment control than PM2.5 treatment, indicating a higher gene expression level when the PM2.5 was not exposed to the lung cell. Figure 4B also showed a substantial disparity in the relative expression of TNF-α, IL-8, and IL-6 genes between no treatment control and 5.0% PM2.5. PM2.5 significantly increased TNF-α and IL-6 gene expression levels up to more than 4-fold. Also, PM2.5 significantly increased IL-8 expression level nearly 2-fold, indicating the notable differences in gene expression due to PM2.5 exposure. IL-2 gene expression level also displayed a disparity between the no-treatment control group and the 5.0% PM2.5 treated group yet showcased a higher relative gene expression level when the cell was not exposed to PM2.5. These outcomes suggest a dependent relationship between PM2.5 exposure and the expression of these inflammatory markers, providing insights into the potential health consequences of elevated PM2.5 levels. Our hypothesis signified that increased PM2.5 treatment would elevate the number of cells with orange, fluorescent marks – dead cells.

## Conclusion

Particulate Matter 2.5 (PM2.5) pollution, composed of fine particles under 2.5 micrometers, was thoroughly studied by assessing cell viability, 3D cultures, and gene expression levels of inflammation markers for its impact on human lung cells. This study demonstrated a clear correlation between the elevation of PM2.5 concentrations and a progressive decrease in cell viability, accompanied by noticeable changes in cell morphology. Moreover, heightened gene expression of inflammatory markers, notably in the 5.0% PM2.5 treatment group, highlights the potential for exacerbated inflammatory responses within human lung cells due to PM2.5 exposure. These findings eventually emphasize the need for stringent measures to curtail PM2.5 emissions and safeguard public health against the associated respiratory risks.

Although the outcomes matched our hypothesis, it was found that this investigation involved several limitations that require future study. The first constraint was the restriction to testing just one type of human lung cell, underscoring the need for a broader sample of human lung cells to validate the research outcomes. Secondly, our study focused only on four inflammation markers, showing the necessity to expand the scope of future research to include a more comprehensive array of inflammation markers. Lastly, the composition of PM2.5, which plays a central role in our investigation, still needs to be thoroughly investigated, particularly regarding heavy metals, bacteria, and harmful chemicals. A deeper exploration of the composition of PM2.5 is crucial to gain insights into the factors contributing to the observed outcomes. Addressing these limitations will be imperative for enhancing the range of our understanding of the research in subsequent studies.

Particulate Matter 2.5 (PM2.5) pollution is a significant environmental concern, comprising fine inhalable particles with diameters of 2.5 micrometers or smaller. When inhaled, these microscopic particles pose serious health risks to humans, leading to various adverse health effects. PM2.5 particles primarily originate from multiple sources, such as combustion processes, industrial activities, and transboundary pollution. This study investigates the impact of PM2.5 on human lung cells and its potential effects on inflammation and cell viability. MRC5 human lung cells were subjected to different concentrations of PM2.5, ranging from 0% to 5%, and incubated for 48 hours. Cell viability was assessed using fluorescent markers, while cell morphology and cell death were observed in 3D cell culture models.

Additionally, gene expression levels of inflammation markers TNF-α, IL-8, IL-6, and IL-2 were analyzed through gel electrophoresis and quantified. The results of this study support the hypothesis that increased concentrations of PM2.5 negatively impact human lung cell viability. A concentration-dependent decrease in cell viability was observed, with higher PM2.5 levels significantly reducing live cells. Also, PM2.5 exposure resulted in alterations in cell morphology, with treated cells appearing scattered and disorganized compared to control cells. This study also demonstrated a clear association between PM2.5 exposure and increased gene expression levels of inflammation markers TNF-α, IL-8, and IL-6. These genes exhibited higher band intensities in the 5.0% PM2.5 treatment group, suggesting a link between PM2.5 exposure and heightened inflammatory responses in human lung cells.

Conversely, the expression level of IL-2 was found to be downregulated in the presence of PM2.5. This downregulation could indicate a suppressive effect of PM2.5 on specific regulatory pathways of the immune response. IL-2 is primarily associated with the activation and proliferation of T cells, and its decrease may reflect a compromised adaptive immune response in the face of PM2.5-induced stress. This downregulation could also respond to the elevated expression of other pro-inflammatory cytokines as the cell attempts to modulate the overall inflammatory response. Further research into the specific mechanisms by which PM2.5 influences IL-2 expression could provide deeper insights into the immunomodulatory effects of PM2.5 and its broader implications for lung health and disease.

Future studies will aim to incorporate a broader range of inflammatory markers beyond TNF-α, IL-8, and IL-6 to provide a more comprehensive understanding of the inflammatory response induced by PM2.5 exposure. By including additional markers, we aim to elucidate the complex and multifaceted inflammatory pathways involved, thereby offering a deeper insight into the pathophysiological mechanisms underlying PM2.5-related lung health issues. This approach will enhance the robustness of our findings and contribute to developing more effective air quality controls and public health initiatives to mitigate the adverse health impacts of air pollution.

Future research should also provide more detailed recommendations for public health policies and directions based on the findings to enhance the impact of this study. Emphasizing the critical need to mitigate PM2.5 pollution through targeted policy interventions and technological innovations will underscore the broader implications of this work. Specific measures such as implementing stricter air quality regulations, investing

in cleaner technologies, and initiating public health programs to monitor and reduce PM2.5 exposure should be advocated.

Future research should address the current study's limitations by including a broader range of lung cell types, incorporating additional inflammatory markers, and conducting a detailed analysis of PM2.5 composition. By expanding the scope to encompass various lung cell types, the research will provide a more comprehensive understanding of the cellular responses to PM2.5 exposure. Additionally, utilizing a wider array of inflammatory markers will offer deeper insights into the complex inflammatory pathways involved. A detailed analysis of PM2.5 composition will help identify specific components contributing to adverse health effects, enhancing the overall understanding of PM2.5's impact on human health. This expanded approach will contribute to more robust and actionable findings, ultimately guiding more effective public health policies and interventions.

In conclusion, this research underscores the adverse effects of PM2.5 pollution on human lung cells, emphasizing the importance of mitigating PM2.5 emissions and implementing measures to reduce human exposure to this hazardous particulate matter. The findings contribute to our understanding of the health risks associated with PM2.5 pollution. They may inform public health and environmental policies to improve air quality and reduce the prevalence of related respiratory diseases.

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