Experimental models and test systems of pollutant interactions (**part of EU action SynAir-G**)

Type of fellowship: Medium-Term Research Fellowship

Home Institution: Qingdao University Qingdao Medical College, China

Host Supervisor: Nikolaos G. Papadopoulos

Host Institution: Allergy Department, 2nd Pediatric Clinic, National and Kapodistrian University Of Athens

Duration: 6 months (13.02.2025-13.08.2025)

Background

The "SynAir-G" hypothesis (https://synairg.eu/):

Asthma, a common chronic inflammatory airway disease primarily divided into extrinsic (allergic) and intrinsic (non-allergic) varieties, is clinically characterized by wheezing, shortness of breath, chest tightness, and coughing often accompanied by varying degrees of expiratory airflow limitation [1]. Allergy, alternatively termed hypersensitivity or allergic reaction, denotes a pathological immune overreaction triggered by constant or recurrent exposure to a specific antigen, potentially resulting in a spectrum of conditions including allergic asthma, allergic rhinitis, and atopic dermatitis [2]. Allergens, which are substances that cause allergic reactions, mainly include pollen, pet hair, house dust mites, and food allergens. Additionally, numerous environmental risk factors such as indoor and outdoor air pollution and climate change can trigger or exacerbate allergic diseases [3]. The World Allergy Organization (WAO) has indicated that the overall prevalence of allergies globally stands at a high 22%, meaning one out of every five individuals suffers from allergic diseases. Between 30-40% of the global population has experienced or is currently experiencing the burden of allergic diseases, with the prevalence continuously rising.

It is projected that by 2050, more than 4 billion people will suffer from diseases such as asthma, allergic rhinitis, and atopic dermatitis, which will have a significant negative impact on health, society, and economic development [4]. Given the impact and severity of these diseases, it is fundamental to better understand their causes and work towards prevention, particularly in childhood [1].

The World Health Organisation (WHO) defines air quality as the level of "contamination of the indoor or outdoor environment by any chemical, physical or biological agent that modifies the natural properties of the atmosphere" [5]. In the last decades, outdoor, or "ambient", air pollution has attracted the most attention in research and media alike. However, nowadays there is increased concern and focus on household and more generally indoor air pollution [6-8]. It is reported that more than 90% of our time is spent indoors, and the impact of indoor air pollution may be particularly significant for certain populations, such as children, and patients with respiratory and allergic diseases, possibly due to higher levels of exposure or increased sensitivity to certain pollutants [9-11]. Among places where children are exposed to indoor pollutants, classrooms rank second only to homes, and classrooms are the only location where public health interventions can be directly implemented. Multiple studies have explored the relationship between indoor air quality and the health of schoolchildren, confirming the presence of various chemical and biological pollutants in classrooms that pose a constant threat to children's physical and mental well-being [12, 13]. Common indoor allergens such as dust mites and mold can trigger allergic or asthmatic symptoms [14]. Additionally, classrooms are also among the primary environments for the transmission of viral infections, making children, particularly those with allergies and asthma, more susceptible to viral respiratory infections [15]. These infections can subsequently spread within the community, affecting elderly individuals and resulting in significantly high morbidity and mortality rates, thereby posing a serious risk to public health [16]. At the same time, viral biological pollutants are also a crucial factor in triggering high reactivity of the respiratory tract to other stimuli, such as allergens or chemical pollutants.

The list of indoor air pollutants that impact on health is long and expanding.

Nevertheless, health effects very often result from repeated, complex exposures to both chemical and biological pollutants; it is clear, but not sufficiently understood, that interactions between different pollutants multiply their health impacts. In the past years, different research studies have already found a link between air pollutants and health, but further research studies is needed to investigate the eventual modification of chemical air pollutants induced by indoor allergens.

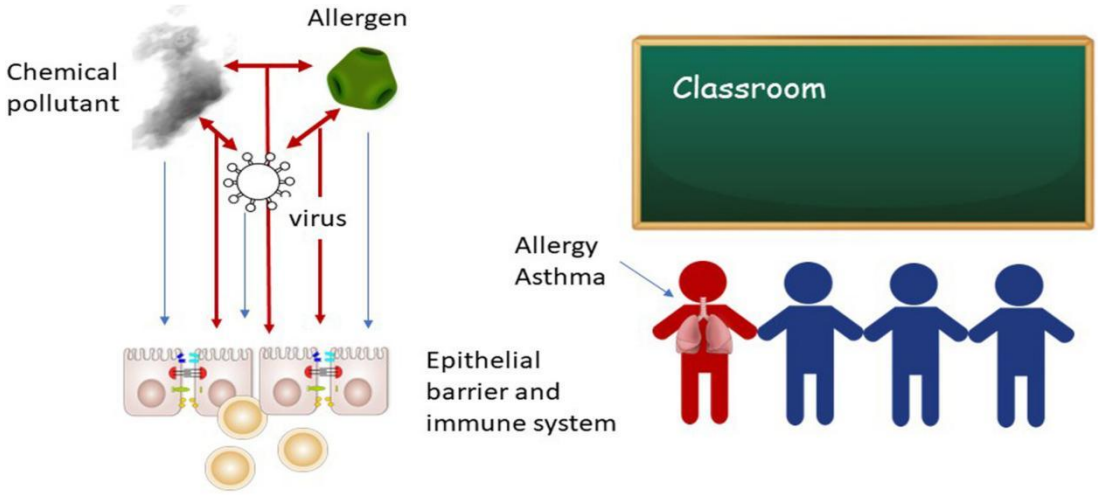

Figure 1 Chemical pollutants, allergens and viruses affect the epithelial barrier and the immune system compromising health. While direct effects (blue lines) have been studied extensively, the interactions between chemical and biological pollutants (red lines) have not been adequately evaluated. Although all may be affected, young children and particularly those with allergy and asthma, are more susceptible to high indoor concentrations of pollutants at school.

Objectives

Understand the dynamics of interaction and synergies between pollutants and airway epithelium, towards informative in-vitro systems. Primary airway epithelial cell model will be developed, based on exposure to relevant pollutant combinations.

Methods

Airway Epithelial cells (cell lines or primary nasal cells), will be exposed to chemical

and biological pollutant combinations. The cell culture system will be tested both in submerged cultures and Airliquid system. The effects of pollutants on cell viability will be assessed using crystal violet colorimetric method.

Results

1. Effect of airborne dust on A549 cells

A549 cells were exposed to increasing concentrations of airborne dust (10–500 µg/mL) for 24 and 48 hours. Cell viability, assessed by crystal violet assay, was not significantly affected at any concentration or time point. However, analysis of the inflammatory marker IL-6 showed a concentration-dependent increase, particularly at 500 µg/mL, after 6, 24, and 48 hours of exposure.

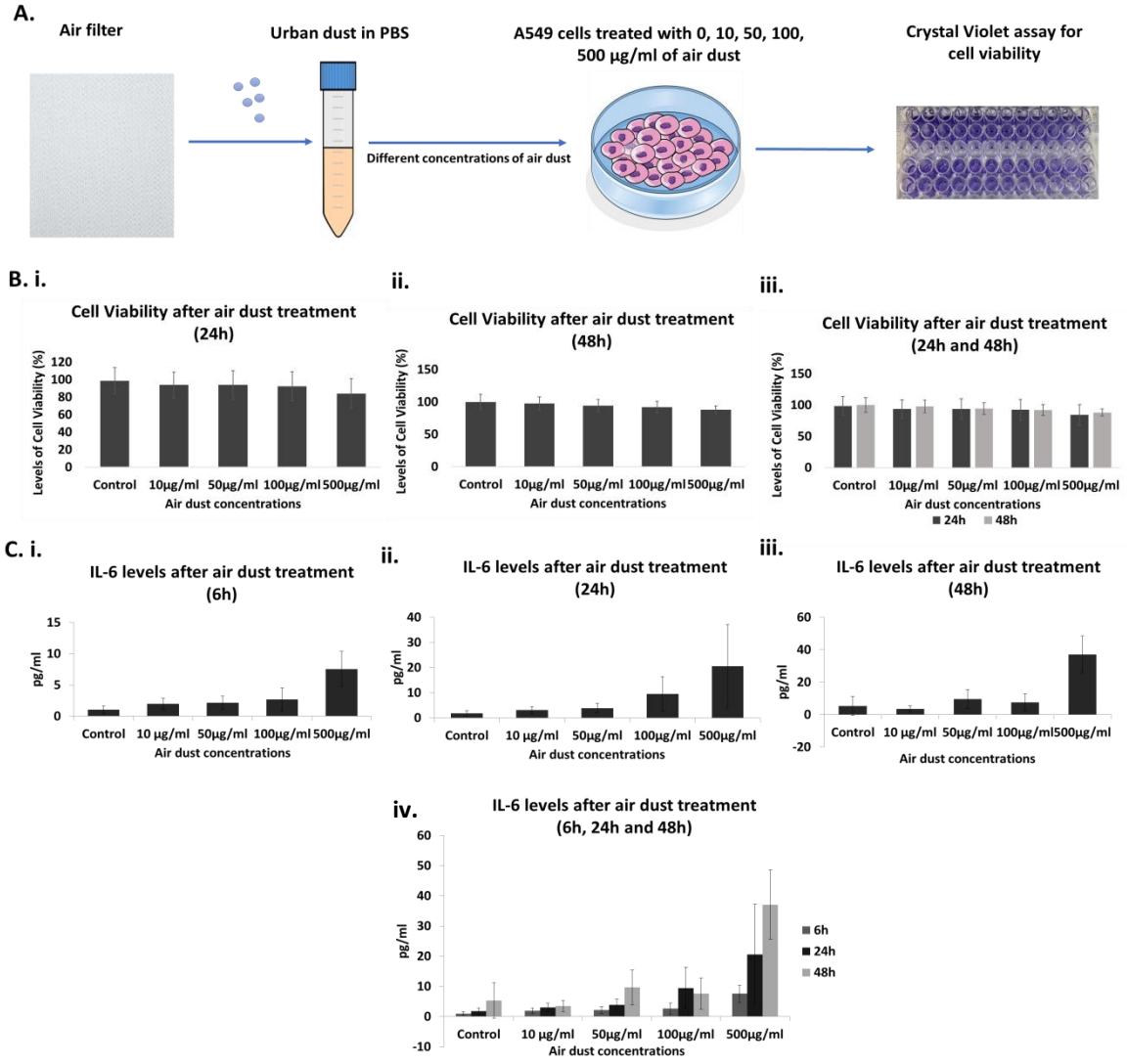

Figure 2. The effect of air dust on A549 cells. A. Air dust was eluted from air filters in

PBS and different dilutions in DMEM with 10% FBS, 1% P/S and 1% non-essential amino acids were followed. A549 cells were treated with different concentrations (10, 50, 100 and 500µg/ml) for 24h and 48h and crystal violet assay was performed to detect the levels of cell viability. B. Detection of the levels of A549 cell viability after exposure to different concentrations of air dust for 24h and 48h. Cell viability levels didn't change significantly after treatment with different concentrations of air dust for i. 24h and ii. 48h. iii. The levels of cell viability didn't affect by different concentrations of air dust and by the time of exposure. C. Detection of the levels of A549 cell viability after exposure to different concentrations of air dust for 6h, 24h and 48h. IL-6 levels increased in correlation with the exposure at higher concentrations of air dust, especially with 500µg/ml compared to control for i. 6h, ii. 24h and iii. 48h. iv. The IL-6 levels were found increased

2. Effect of PM2.5 on A549 cells

Exposure of A549 cells to PM2.5 (10–100 µg/mL) for 24 hours resulted in a significant decrease in cell viability at the highest concentration (100 µg/mL), as shown by crystal violet staining. Morphological changes indicative of cytotoxicity were also observed at this concentration.

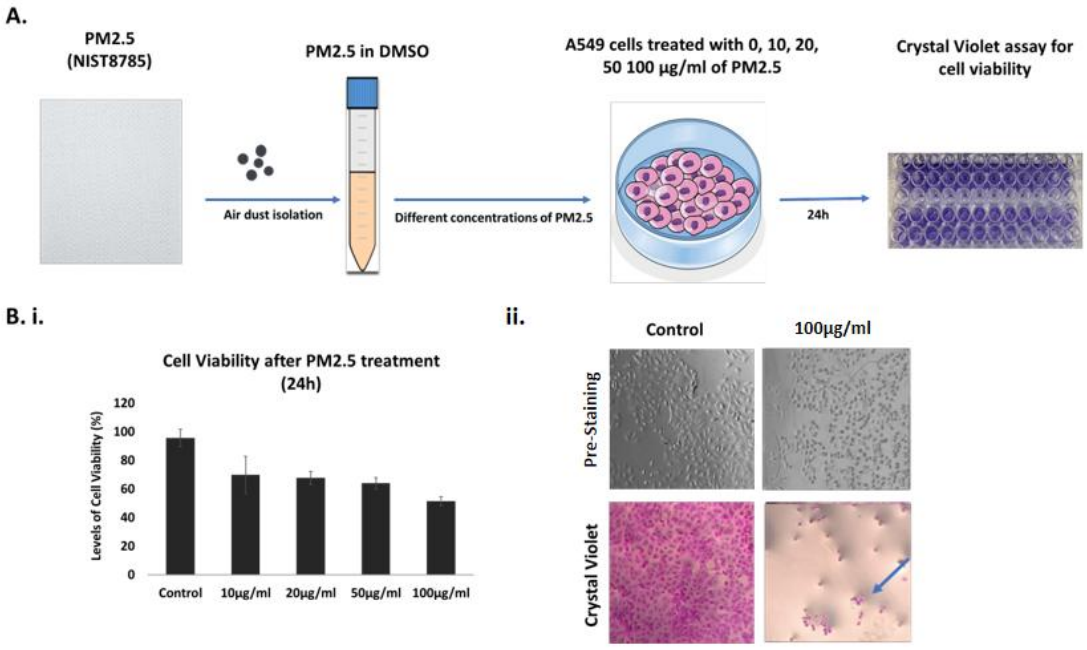

Figure 3. The effect of PM2.5 on A549 cells. A. PM2.5 were eluted from filters in DMSO and different dilutions in DMEM with 10% FBS, 1% P/S and 1% non-essential amino acids were followed. A549 cells were treated with different concentrations of PM2.5 (10, 20, 50 and 100μg/ml) for 24h and crystal violet assay was performed to detect the levels of cell viability. B. i. Quantitative analysis of cell viability after 24h exposure to PM2.5. ii. Representative images of crystal violet staining of A549 cells. The higher concentration of PM2.5 (100μg/ml) significantly decreased the cell viability compared to control (untreated cells-0 μg/ml PM2.5). Original magnification 10x. Blue arrows indicate the change of cell morphology after the treatment with PM2.5.

Acknowledgments:

I am deeply thankful to my host supervisor, Prof. Nikolaos G. Papadopoulos, for providing me with this invaluable opportunity and for his mentorship. I also extend my appreciation to the members of the 2nd Pediatric Clinic at the National and Kapodistrian University of Athens for their assistance and collaboration.

Conclusion and Personal Reflection:

This six-month fellowship provided an invaluable opportunity to contribute to the EU SynAir-G project and significantly enhanced my practical research skills. Under the guidance of Prof. Papadopoulos and his team, I successfully established and utilized in vitro models of airway epithelium (A549 cell line) to assess the biological impact of common indoor pollutants. This experience profoundly deepened my understanding of environmental health mechanisms in allergic diseases. The skills acquired in experimental design, data analysis, and scientific collaboration are directly transferable to my ongoing research in pediatric allergy in China. I am confident that the insights gained will positively influence my future clinical and research endeavors, enabling me to contribute more effectively to addressing the global challenge of environmentally triggered respiratory diseases.

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