Response of Pulmonary Cells to Bolus and Incremental Doses of Engineered Nanoparticles

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Introduction

Chronic respiratory diseases, including chronic obstructive pulmonary disease (COPD), asthma, interstitial lung diseases, and pneumoconiosis, are the third leading cause of death worldwide, accounting for 4 million deaths in 2019. Inhaled particulate matter (PM) from environmental and occupational sources introduces exogenous pro-oxidants to the respiratory space, increasing the risk of pulmonary disease development and exacerbation.

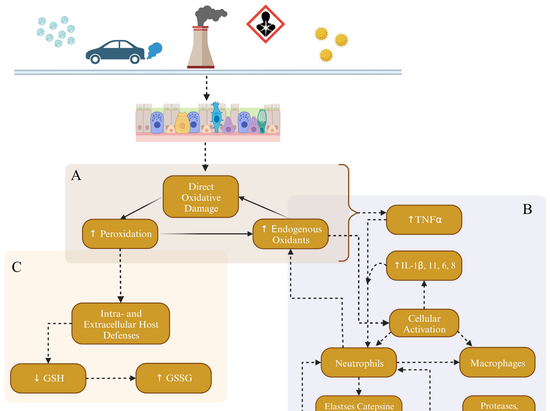

Figure 1. The multi-level cycle of cellular responses to exogenous pro-oxidant exposure. A) Level 1 represents the interaction of exogenous pro-oxidants with the pulmonary cell space. B) Cellular activation resulting from endogenous oxidants and the release of inflammatory mediators triggers an oxidative cycle in Level 2. C) Level 3 involves host defense mechanisms including enzymatic and nonenzymatic antioxidant systems.

Objectives

This in vitro study aims to simulate short- and long-term inhalation exposures to particulate matter while considering pulmonary clearance mechanisms. Human lung epithelial cells were exposed to bolus and incremental doses of engineered nanoparticles over 8 hours to mimic occupational exposures in 8-hour workdays. The oxidative stress response of the cells was characterized via changes in viability, inflammatory response, and antioxidant defense capacity.

Methods

Material Characterization

The physiochemical properties of titanium (IV) oxide (TiO_2) nanopowder (nominal particle size of <100 nm; Sigma Aldrich) were characterized using DLS, zeta potential (Zetasizer Nano ZS, Malvern), SEM (Axia ChemiSEM, Thermo Scientific), and XRD (Empyrean, Malvern).

Cell Culture

A549 cells (ATCC) were cultured in F-12K medium supplemented with fetal bovine serum (10% v/v) and penicillin-streptomycin (1% v/v) and incubated at 37°C humidified atmosphere with 5% CO_2. Experiments were conducted in passages 8-15. Cells were seeded in 24-well plates at a seeding density of $1x10^4$ cells/cm^2 and incubated for 48 h prior to experiments.

Methods

Exposure Conditions

TiO_2 particles were suspended in phosphate buffer solution containing Tween-20 (PBST, 0.05% v/v Tween-20) at a concentration of 1 mg/ml. Particles were washed by performing 4 rounds of vortexing, bath sonication, and centrifugation with PBST replaced after each round. Serial dilutions were performed to obtain particle suspensions at concentrations of 0.019, 0.19, 1.9, 19, and 190 mg/ml. Exposure concentrations were determined by scaling the legally permissible airborne exposure concentration to an inhalation equivalent of one well of a 24-well plate.

Particles were administered to cell populations in 8-h bolus or incremental schedules with medium replaced 10 h or 2 h after the last exposure. Cell responses were evaluated following exposures including cell viability, inflammatory cytokine secretion, and ratio of reduced to oxidized glutathione.

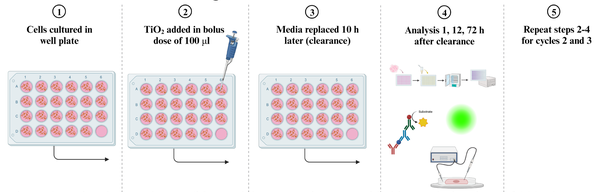

Figure 2. Bolus dose regimen. TiO_2 particle suspension was added in one bolus dose of 100 µl. Media was replaced 10 h later to represent pulmonary clearance. Cell responses were evaluated 1, 12, and 72 h after clearance.

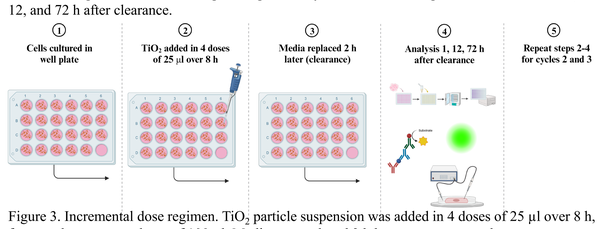

Figure 3. Incremental dose regimen. TiO_2 particle suspension was added in 4 doses of 25 µl over 8 h, for a total exposure volume of 100 µl. Media was replaced 2 h later to represent pulmonary clearance. Cell responses were evaluated 1, 12, and 72 h after clearance.

Results

Physiochemical Properties of TiO_2 Nanopowder

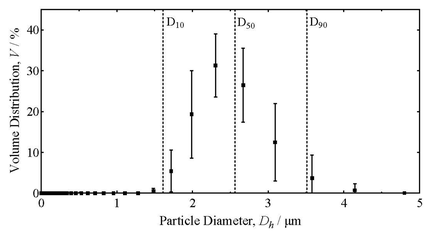

Figure 4. An average hydrodynamic diameter of 2.5 µm was determined using DLS. Analysis of SEM imaging determined average individual particle diameters of 100 nm, noting a large degree of agglomeration.

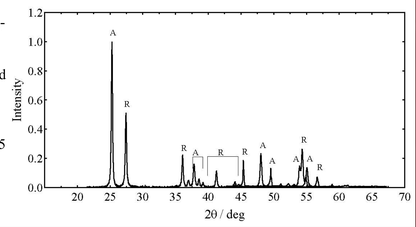

Figure 5. XRD patterns of TiO_2-NP showing anatase (A) and rutile (R) peaks. The sample consisted of 54 wt% anatase and 46 wt% rutile phases. [Peak references: Meagher E.P., and A.L. George (1979). Canadian Mineralogist 17:77-85 and Wyckoff R.W.G. (1963). Crystal Structures 1:253-254]

Results

Effects of TiO2 Bolus Dose Administration

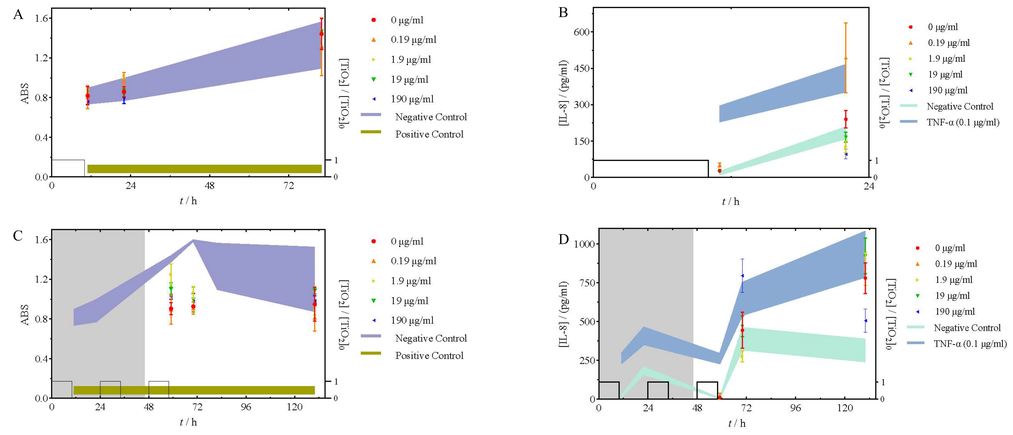

Figure 6. Viability and inflammatory response to bolus dose schedule after A, B) 1 bolus dose and C, D) 3 bolus doses. A, C) Viability results of the exposure concentrations are presented as averages of absorbance measurements with error bars representing standard deviation for replicates of n = 3. The negative and positive controls are presented as swathes that encompass respective average absorbance values and standard deviations, n = 3. B, D) IL-8 secretion in culture. Results of the exposure concentrations are presented as averages of interpolated supernatant cytokine concentrations with error bars representing standard deviation for replicates of n = 2. The negative and positive controls are presented as swathes that encompass respective average cytokine concentrations and standard deviations, n = 2.

Effects of Incremental Dose Administration

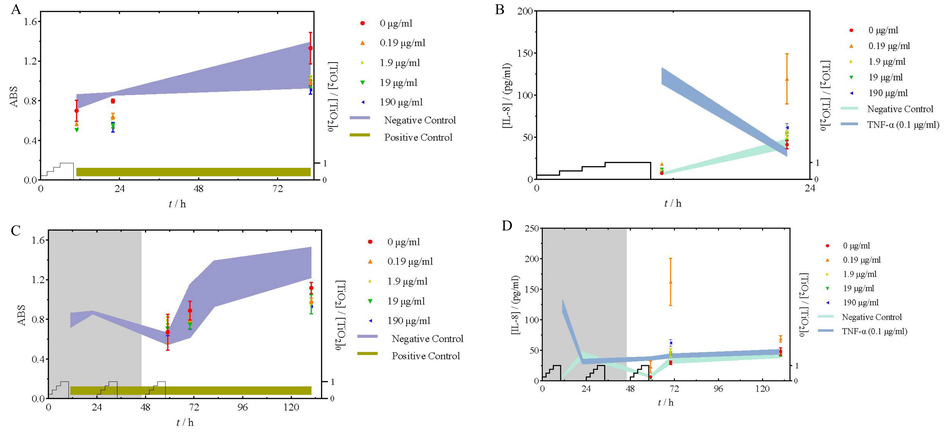

Figure 7. Viability and inflammatory response to incremental dose schedule after A, B) 1 dose and C, D) 3 doses. A, C) Viability results of the exposure concentrations are presented as averages of absorbance measurements with error bars representing standard deviation for replicates of n = 3. The negative and positive controls are presented as swathes that encompass respective average absorbance values and standard deviations, n = 3. B, D) IL-8 secretion in culture. Results of the exposure concentrations are presented as averages of interpolated supernatant cytokine concentrations with error bars representing standard deviation for replicates of n = 2. The negative and positive controls are presented as swathes that encompass respective average cytokine concentrations and standard deviations, n = 2.

Conclusions

While in vitro cell systems are extensively used in toxicological studies, very few have been applied to repeated and prolonged exposures and incorporate pulmonary clearance mechanisms. This is the first study to our knowledge that attempts to incorporate pulmonary clearance in an in vitro cell culture exposure system.

This study demonstrated the effects of bolus and incremental dose regimens of TiO_2 in human pulmonary cells in short-term and prolonged schedules. While viability remained relatively unimpacted by particle exposures, elevated inflammatory responses were observed over time. Additionally, significantly elevated inflammatory responses were observed in bolus-dose scenarios compared to repeat-dose regimens.

This work will be continued to evaluate longer-term exposures and the downstream impacts of different environmental and occupation particulate matter.

Acknowledgments

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