

RESEARCH ARTICLE

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Evaluating the toxicity of polystyrene micro- and nanoplastics in human bronchial epithelial cells: differences and challenges using aerosol and suspension exposures

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ABSTRACT

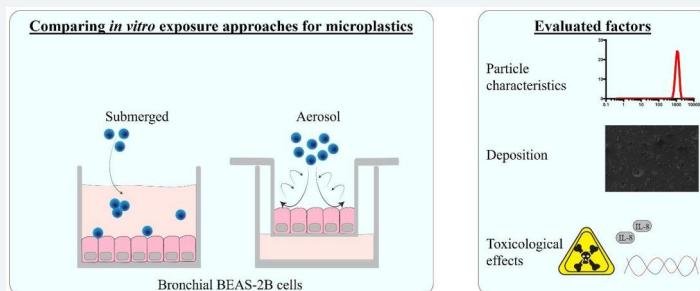
Objective: Studies investigating toxicity of airborne micro- and nanoplastics (MNPs) are mostly based on conventional submerged cell cultures while limited studies utilize air-liquid interface (ALI) systems. Inherent differences in these culture/exposure modalities, such as particle behavior and deposited dose, likely affect cellular responses. To investigate this, we exposed submerged human bronchial epithelial cells (BEAS-2B) to polystyrene (PS) MNPs vs. aerosol exposure of ALI cultures.

Methods: First, submerged bronchial epithelial cells (BEAS-2B) were exposed to suspensions of PS particles (50 nm or 1 μ m) in four different applied concentrations (0.79–50 μ g/cm²; 24 h). Second, BEAS-2B cells were cultured at ALI and exposed in a cloud system to PS 1 μ m particles (deposited dose: 55.4 μ g/cm²; 24 h). Toxicity readouts focused on cytotoxicity (LDH release), inflammation (IL-8 release and transcriptional activation of inflammatory genes), and oxidative stress (DCFH-DA assay, antioxidant gene expression, and assessment of reduced/oxidized glutathione).

Results: In both models, PS exposure did not induce cell death, or an antioxidant response. However, NF- κ B transcriptional activity was strongly upregulated in submerged cells in response to both sizes of PS particles in a dose-dependent manner. Gene expression of *CXCL1*, *CXCL2*, and *CXCL8* increased up to 7-fold after PS microplastic exposure (50 μ g/cm²) in the submerged model (which was less pronounced in response to PS nanoplastics) and 2-fold in the ALI model. In contrast, IL-8 secretion increased 1.6-fold for the ALI, but not the submerged model.

Conclusions: Overall, both exposure modalities revealed an inflammatory response toward PS MNPs although with differences, likely due to significant differences in deposited dose.

GRAPHICAL ABSTRACT



PLAIN LANGUAGE SUMMARY

Very small plastic particles, called microplastics and nanoplastics (MNPs), are present in the air and can be inhaled into the lungs. To study possible health effects, scientists often expose lung cells to these particles in liquid (submerged) culture systems. However, this does not closely match how people are exposed through breathing. This study used a traditional liquid-based method and a more realistic system that exposes lung cells to plastic particles through the air. More specifically, human lung cells were exposed to polystyrene (PS) plastic particles of different

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sizes using either liquid exposure or an air–liquid interface system, where particles were delivered as an aerosol, similar to inhalation. The researchers looked for signs of cell damage, inflammation, and oxidative stress. The results showed that the plastic particles did not kill the cells and did not cause oxidative stress in either exposure method. However, both methods caused signs of inflammation, which is a normal response of the body to foreign substances. In the liquid-based system, inflammation-related genes were strongly activated. In the air-exposed system, gene activation was weaker, but cells released higher levels of an inflammatory signaling protein. Importantly, the air-based exposure better reflects how people actually breathe in particles and avoids some problems linked to liquid-based testing. This study shows that there are differences in cellular responses to MNPs depending on the dose, particle behavior and culture system, which are all connected to each other.

Introduction

In recent years, environmental pollution by microplastics ($< 5\text{ }\mu\text{m}$) and nanoplastics ($< 1\text{ }\mu\text{m}$) (MNPs) has emerged as a significant global concern due to their pervasive presence and potential health implications. Airborne MNPs originate from a variety of sources, including tire wear, agriculture, and industrial processes (Zhao et al. 2023). Humans are constantly exposed to airborne MNPs, especially indoors, with most recent estimated exposure levels being around 68,000 MPs/d (Yakovenko et al. 2025). MNPs smaller than $10\text{ }\mu\text{m}$ can enter the airways where they can encounter the bronchial epithelium. This critical barrier in the respiratory system plays a pivotal role in protecting against inhaled particles, pathogens, and pollutants (Braakhuis et al. 2014). MNPs of various polymers have been detected in all areas of the lung, as well as in other parts of the human body (Amato-Lourenço et al. 2021; Jenner et al. 2022). Understanding the interactions between inhaled MNPs and bronchial epithelial cells (BEAS) is essential for assessing potential health risks.

As use of environmentally relevant MNPs in toxicity research up till now has been limited due to lack of commercial availability and technical difficulties in generating these MNPs, most studies thus far have used polystyrene (PS) as a model MNP. These mono-dispersed particles with well-defined sizes are suitable to study correlations between size and particle toxicity (Zhang et al. 2022; Luo et al. 2025). In addition, the uniform spherical shape of these model MNPs allows to quantify the dose in number or relative surface area, considered a more biologically relevant metric than mass-based (Schmid and Stoeger 2016). Indicative of the potential toxicity of PS MNPs, intratracheal instillation of mice with PS for two weeks resulted in inflammatory cell infiltration in the lung and increased levels of interleukin-6 in bronchoalveolar lavage fluid (Danso et al. 2022). Due to ethical concerns and translational challenges in predicting the

human toxicity response toward environmental pollutants, the field is moving away from *in vivo* experiments toward new approach methodologies, including *in vitro* models. *In vitro* models allow researchers to study the effects of MNPs on key cellular events upstream of tissue injury, most important being cytotoxicity, inflammation, and oxidative stress (Halappanavar and Mallach 2021). For example, 24 h exposure to 1 mg/cm^2 PS microplastics ($1.7\text{ }\mu\text{m}$) was previously shown to induce cytotoxicity, while also inducing intracellular reactive oxygen species (ROS) in bronchial epithelial cells (BEAS-2B) (Dong et al. 2020).

Despite the growing number of *in vitro* studies performed in the field of MNPs, studies focusing on the effects of airborne MNPs are still very much restricted to submerged exposures. However, the air liquid interface (ALI) culture system provides a useful tool and more physiologically relevant system to examine the response of epithelial cells of the airways and lungs to airborne pollutants. This system has been used for toxicological research of metal nanoparticles, as well as for diesel exhaust particles and particulate matter (Hufnagel et al. 2020; Kaur et al. 2022). Studies comparing exposure methods using non-plastic particles indicate exposures performed at ALI may substantially differ from submerged exposures which could be related to inherent model differences, dosimetry, and particle behavior/characteristics (Kaur et al. 2022; Silva et al. 2023; Allouche et al. 2025). However, these studies reported variable results, and outcomes might differ per type of airborne pollutant. Before selecting the most suitable exposure and culture system, it is essential to evaluate the influence of exposure method on MNP characteristics and subsequent cellular responses.

This study aimed to assess the biological responses of human BEAS to MNPs in a submerged – vs ALI setting. We used PS as model MNP and investigated the effect of exposure setting on particle characteristics,

deposition/dose, and toxicological effects. As toxicological endpoints we selected cytotoxicity, inflammation and oxidative stress, as based on literature, these key events are the ones most likely to occur after MNPs exposure. In addition, we describe the considerations and challenges of implementing both exposure methods for MNPs studies, which will help guide future research efforts in this emerging field.

Materials and methods

Preparation of particle suspensions

Polystyrene Polybead[®] Microspheres (PS) with a diameter of 50 nm and 1 μm were obtained in watery suspension (Polysciences, Warrington, PA, USA). For submerged exposures, particle stocks were pre-diluted in 0.5% Bovine Serum Albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA), to increase steric repulsion and prevent agglomeration, and then further diluted to desired particle concentration while reaching a final concentration of 0.05% BSA, 1% heat inactivated fetal bovine serum (FBS), and 100 U/mL Penicillin/100 $\mu\text{g/mL}$ Streptomycin in Dulbecco's Modified Eagle Medium (DMEM) (all derived from Thermo Fisher Scientific, Waltham, MA, USA). For ALI exposures, PS stock was diluted in a mixture of MilliQ-water (MQ) and Dulbecco's Phosphate-Buffered Saline (dPBS; Thermo Fisher Scientific), to a final concentration of 14 mg/mL.

Characterization of particle suspensions

Scanning electron microscopy, combined with energy dispersive X-ray spectroscopy (SEM/EDX) was used, as previously described, to confirm elemental composition and shape of the particles (Gosselink et al. 2025). Dynamic Light Scattering (DLS) was performed using a Zetasizer nano ZS (Malvern Panalytical, Malvern, UK) to determine particle size distributions in diluted stock suspensions in both MQ and (submerged) exposure medium (0.1–0.5 mg/mL). The refractive index for PS was set at 1.59 (Zhang et al. 2020). The average spectrum from three measurements was used to calculate the hydrodynamic diameter. The same machine was used to determine the zeta-potential, indicating surface charge of PS particles in MQ (equilibration time 120 s).

BEAS-2B cell culture and exposure

Human BEAS-2B, were obtained from American Type Culture Collection and cultured in complete

medium (DMEM supplemented with 10% FBS and 100 U/mL Penicillin/100 $\mu\text{g/mL}$ Streptomycin) in a humidified incubator with 5% carbon dioxide at 37 °C. Medium replacement was performed approximately every 2 d and cells were passaged before confluency. For submerged exposures, cells were seeded in full medium 24 h prior to exposure, in a 48-well plate (30,000 cells/well). For ALI exposures, 55,000 cells were seeded 5 d prior to exposure in complete medium on the apical side of 12-well 12-mm transwells with 0.4- μm pore polyester membrane cell culture inserts (Corning Costar, New York, NY, USA). Three days later, complete medium was refreshed. The next day, 24 h before exposure, cells were airlifted by removing the apical medium and replacing the basolateral medium by 1 mL reduced medium (DMEM supplemented with 1% heat-inactivated FBS and 100 U/mL Penicillin/S, i.e. reduced concentration of FBS). All exposures were performed in three or four biological replicates, each including three technical replicates.

Submerged exposure

Cells were exposed to PS 50 nm or PS 1 μm in different doses: 0.79 $\mu\text{g/cm}^2$, 2.38 $\mu\text{g/cm}^2$, 7.14 $\mu\text{g/cm}^2$, and 50 $\mu\text{g/cm}^2$ for 24 h. Exposures occurred in particle suspensions (paragraph 'Preparation of particle suspensions') of 200 μL in 96-well plates (DCFH-DA assay, NF- κB and AP-1 transcriptional activity assay), 400 μL in 48-well plates (cytotoxicity, IL-8 ELISA, gene expression measurements) or 2 mL in 6-well plates (GSH:GSSG assay). A vehicle control, (DMEM with 1% FBS, 100 U/mL Penicillin/100 $\mu\text{g/mL}$ Streptomycin, 0.05% BSA) as well as a positive control for inflammation (Tumor Necrosis Factor- α [TNF- α]; 50 ng/mL, Thermo Fisher Scientific), was included in all experiments. For better comparison, we modeled the deposition of the 1 μm PS particles in the submerged system. For this, we applied the RiskGONE *in vitro* dosimetry web application, which is based on the Harvard DG model developed by DeLoid et al. (2015). Solvent parameters of the exposure medium were: media density = viscosity = (Gosselink et al. 2025). The effective density in DMEM 1% FBS, as measured using the volumetric centrifugation method (DeLoid et al. 2014) was provided by H. Dusza: 1.0259 g/cm³ (Zheng et al. manuscript in preparation). The following additional parameters were used in the model: polymer density of PS = 1.05 g/cm³, suspension column height = 3.64 mm, height of sub-compartment = 0.02,

centrifugation = 1 (gravity), total simulation time = 24 h, and time interval for simulation = 1 s. The resulting estimated deposited dose of PS 1 μm was reported in particle mass per unit surface area ($\mu\text{g}/\text{cm}^2$), as well as fraction of the applied dose.

ALI exposure

The Vitrocell® Cloud alpha move system (Vitrocell, Waldkirch, Germany) was used for ALI exposures, equipped with a vibrating mesh nebulizer with droplet mass mean aerodynamic diameter ranging from 4.0 to 6.0 μm . During exposure, cells were maintained at 37 °C and provided with reduced medium *via* the basolateral compartment. The aerosol was generated by nebulizing 500 μL of the (14 $\mu\text{g}/\text{mL}$) working suspensions, using a flow rate around 400 $\mu\text{L}/\text{min}$. Following nebulization, the aerosol was allowed 6 min to settle and subsequently evacuated for 3 min using the PowerVent system (Vitrocell). Each culture was exposed three times: either three times with MQ-PBS (vehicle control) or one-time MQ-PBS and two times with PS suspensions, aiming to match the nominal concentration (50 $\mu\text{g}/\text{cm}^2$) of the highest dose implemented for submerged exposures. A quartz crystal microbalance (QCM; Vitrocell) was embedded in the cloud chamber for determination of the deposited mass. Directly after exposure, cells were placed in new plates with fresh reduced medium containing at the basal side and kept at the ALI for 24 h in the incubator.

Cytotoxicity assay

To determine cytotoxicity, Lactate dehydrogenase (LDH) analysis was performed according to manufacturer's specifications (Cytotoxicity Detection Kit (LDH); Roche, Basel, Switzerland). Of 24 h after exposure an apical wash (dPBS) was collected for the ALI exposures, as well as the supernatant of the submerged exposures. Absorbance values were measured at 492 nm (reference wavelength 650 nm) with a SpectraMax iD3 Multi-Mode Microplate reader (Molecular Devices, San Jose, CA, USA) and corrected for medium/dPBS background. As a positive control (LDH max), cells were exposed to 2% Triton-X-100 (Sigma-Aldrich) for 10 min. Cytotoxicity was expressed relative to the LDH max.

IL-8 ELISA

As indicator of pro-inflammatory response, interleukin-8 (IL-8) protein secretion was quantified in the

supernatant (submerged exposures) or apical and basolateral compartments (ALI exposures) using the IL-8/CXCL8 ELISA DuoSet kit (R&D Systems, Minneapolis, MN, USA). To remove debris and floating cells, supernatant or apical wash was centrifuged at 4000 rcf for 10 min at 4 °C. Absorbance measurements were performed at 450 nm (reference wavelength 570 nm) using the SpectraMax iD3 Multi-Mode Microplate reader. Absorbance values were corrected for a blank control (exposure medium, i.e. DMEM or PBS) and IL-8 protein levels were quantified based on the standard curve, using a four-parameter logistic regression curve.

Quantitative polymerase chain reaction (qPCR)

TRIzol reagent (Invitrogen, Waltham, MA, USA) was used to extract RNA, according to manufacturer's instructions. Isolations, cDNA synthesis, quantitative polymerase chain reaction (qPCR) as well as analysis has been performed as previously described (Gosselink et al. 2025). qPCR was performed on the CFX Opus 384 Real-Time PCR System (Bio-Rad, Hercules, CA, USA) and analyzed with the CFX Maestro software version 2.3 (Bio-Rad) and LinRegPCR software 2014. Gene expression levels of target genes were normalized using the geometric mean of four housekeeping genes (beta-actin (*ACTB*), beta-2 microglobulin (*B2M*), Cyclophilin A (*CYPA*), and ribosomal protein L13 A (*RPL13 A*)) and subsequently expressed as fold change to vehicle control. Primers are listed in [Supplementary Table S1](#).

DCFH-DA assay

To detect general intracellular ROS after particle exposure, a dichlorofluorescein diacetate (DCFH-DA) assay was performed. First, BEAS-2B cells were seeded in a 96-well plate at a seeding density of 2.5×10^5 cells/well. After 24 h, the cells were incubated for 40 min with 10 μM DCFH-DA (Sigma-Aldrich) in reduced medium without phenol red. Thereafter, cells were exposed to either PS MNPs or vehicle control, in phenol red-free reduced medium. Of 1 mM H_2O_2 served as positive control. Fluorescence was measured after 24 h using a SpectraMax iD3 Multi-Mode Microplate reader (excitation 485 nm and emission 535 nm) and expressed as fold change to the vehicle control.

Measurement of GSH and GSSG levels

Another useful indicator for oxidative stress is the intracellular reduced glutathione (GSH)/oxidized

glutathione (GSSG) ratio, which was determined after PS exposure exactly as previously described (Rahman et al. 2006; Gosselink et al. 2025). Total glutathione (GSH + GSSG) and the oxidized form (GSSG) were quantified in two parallel reactions whereafter reduced glutathione levels could be quantified. Data are presented as GSH:GSSG ratios.

NF- κ B and AP-1 transcriptional activity assay

To study the transcriptional activity of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and Activator protein-1 (AP-1) inflammatory pathways, we performed exposures on BEAS-2B reporter cells, transduced with a Luciferase reporter driven by NF- κ B (RelA/p50) or AP-1 (Fos/Jun) transcription factor binding elements, as described earlier (Housmans et al. 2023; Gosselink et al. 2025). In short, 24 h before exposure to PS MNPs, transduced BEAS-2B cells were seeded in a 96-well plate. Of 24 h after exposure, cells were washed with PBS and lysed in MQ. A Tristar 2 injection system (Berthold Technologies, Bad Wildbad, Germany) was used to add Nano-Glo[®] reagent to each well. Luminescence was quantified using the Tristar2 LB942 multi-mode plate reader (Berthold Technologies).

Statistical analysis

Data are shown as the mean $\pm$ standard deviation for three independent experiments. Statistical analysis was performed with GraphPad Prism version 10.3.1 (GraphPad Software, San Diego, CA, USA). The Shapiro–Wilk test was used to check for normality. For dose-response data statistical differences between vehicle and treatment conditions were tested using an ordinary one-way ANOVA with Dunnett's multiple comparisons test (normally distributed data) or a Kruskal–Wallis test (non-normally distributed data). For single-dose exposures, a two-tailed unpaired *t* test was used to test statistical differences. Values of $p < 0.05$ were considered significant.

Results

Particle characteristics

PS particles displayed a spherical morphology, as assessed by SEM (Figure 1(A,B)). Diluted stock suspensions of PS particles were characterized using DLS which revealed an average hydrodynamic diameter in the same size range as their primary particle size; 68 ± 0.47 nm and 1024 ± 16 nm. The low polydispersity

index for both PS nanoparticles (0.045 ± 0.01) and PS microparticles (0.053 ± 0.04), and narrow size distribution spectra indicated suspensions were highly mono-dispersed (Figure S1). The zeta potential in MQ was -34.0 mV ± 1.1 and -31.1 mV ± 1.8 for the PS nanoparticles and PS microparticles suspensions, respectively, indicating a strong electrostatic repulsion between PS particles in MQ. To get more insight into PS particle behavior in suspension we also measured the particle size in exposure medium. In exposure medium, in the presence of BSA and FBS, a broader size distribution of PS nanoparticles was observed (196 ± 31.49 nm), whereas the size distribution of PS 1 μ m remained similar to the distribution in MQ (1057 ± 77.88 nm) (Figure S1).

Particle deposition

For submerged exposures with PS micro- and nanoparticles, the applied dose ranged from 2.2 μ g/mL to 137.5 μ g/mL (48-well format) and 1.3 μ g/mL to 80 μ g/mL (96-well format). The narrow size distributions and spherical shape of the PS particles enabled us to accurately transfer the applied mass-based doses toward other relevant dose metrics, including particle number and surface area (Table 1). Representative pictures illustrative of the deposition of the 1 μ m particles relative to cell surface area are depicted in Figure S3(A). The 50 nm particles were too small to be visualized with light microscopy. The theoretical delivered dose in all submerged exposures in case of 100% deposition efficiency would be 0.79 μ g/cm² – 50 μ g/cm². Importantly, to more accurately estimate the effective dose of 1 μ m PS particle in the submerged culture condition, we modeled the deposited dose using the RiskGONE *in vitro* dosimetry web application. This revealed that after 24 h, in the submerged system, only approximately 8% of the applied dose was deposited, resulting in an effective dose of approximately 4.1 μ g/cm² for the highest dose (Figure S6).

For the ALI system, the deposited dose of 1 μ m particles was 55.43 μ g/cm², as assessed by QCM (Figure S4(A,B)). Considering a theoretical maximal deposited mass of 69.8 μ g/cm², the overall deposition efficiency was 79%. SEM pictures from grids revealed the exposure surface was relatively homogeneously covered by PS particles and PBS salts, with some agglomerated particles present (Figure 1(C,D)).

In summary, the exposure method influenced deposition and particle behavior, in which nebulization is preferred over the submerged exposure

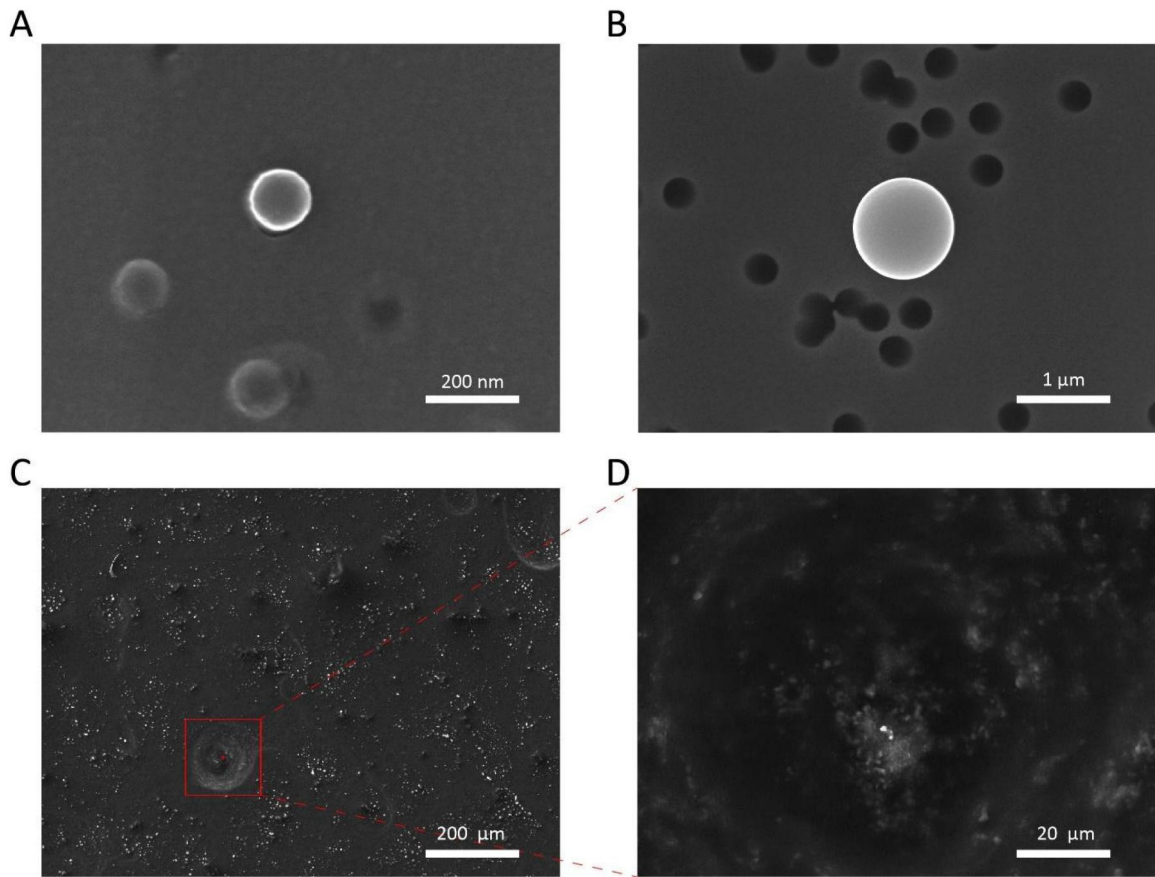


Figure 1. Characterization of polystyrene particles by scanning electron microscopy (SEM) Representative secondary electron images of polystyrene (PS) nano- (A) and microparticles (B) in diluted stock suspensions. SEM grids placed in the aerosol chamber during nebulization were analyzed to assess the distribution of the deposited PS microparticles and salt particles (phosphate-buffered saline) in air-liquid interface exposures (C). Figure (D) is the high-magnified SEM image of figure C, revealing clusters of spherical PS particles.

Table 1. Different dose metrics for polystyrene particles in submerged exposures.

	PS 50 nm				PS 1 μm			
Mass (μg/mL)	2.2	6.6	19.6	137.5	2.2	6.6	19.6	137.5
Number (#particles/mL)	3.20E + 10	9.60E + 10	2.85E + 11	2.00E + 12	3.18E + 06	9.53E + 06	2.83E + 07	1.99E + 08
Surface area (μm ² /mL)	2.51E + 08	7.54E + 08	2.24E + 09	1.57E + 10	1.16E + 07	3.49E + 07	1.04E + 08	7.28E + 08
Nominal dose (μg/cm ²)	0.79	2.38	7.14	50	0.79	2.38	7.14	50

Applied doses for polystyrene (PS) based on particle number and surface area were derived from the mass-based dose, taking into account the particle diameter (0.05 and 1.08 μm as derived by Scanning Electron Microscopy) and the polymer density of PS (1.05 g/cm³). The nominal doses of PS particles (μg/cm²) were calculated with the exposure volume (400 μL) and surface area of the cell culture system (1.1 cm²), assuming 100% deposition efficiency.

method, since the delivered dose is not influenced by media interactions and can be quantified more accurately. In the subsequent described results, for submerged exposures we refer to the nominal dose in μg/cm², while for ALI we report the deposited dose (55.43 μg/cm²).

Effects of polystyrene particles on submerged BEAS-2B cells

Assessment of cytotoxicity as revealed by release of LDH in the cell culture supernatant showed absence

of any impact on cell viability in submerged exposures (Figure S3(B)). Next, we assessed the impact of these PS particles on cellular oxidant/antioxidant status. As illustrated in Figure 2(A), using H₂O₂ as a positive control (FC = 2.90, $p = 0.001$), 50 μg/cm² PS 50 nm or PS 1 μm led to a small, but significant reduction in intracellular ROS, as assessed by the DCFH assay (FC = 0.53, $p = 0.03$, and FC = 0.33, $p < 0.001$, respectively). In an effort to further characterize potential impact on relevant cellular antioxidant defense systems, we assessed abundance of the total levels of glutathione (GSH) vs. its oxidized form,

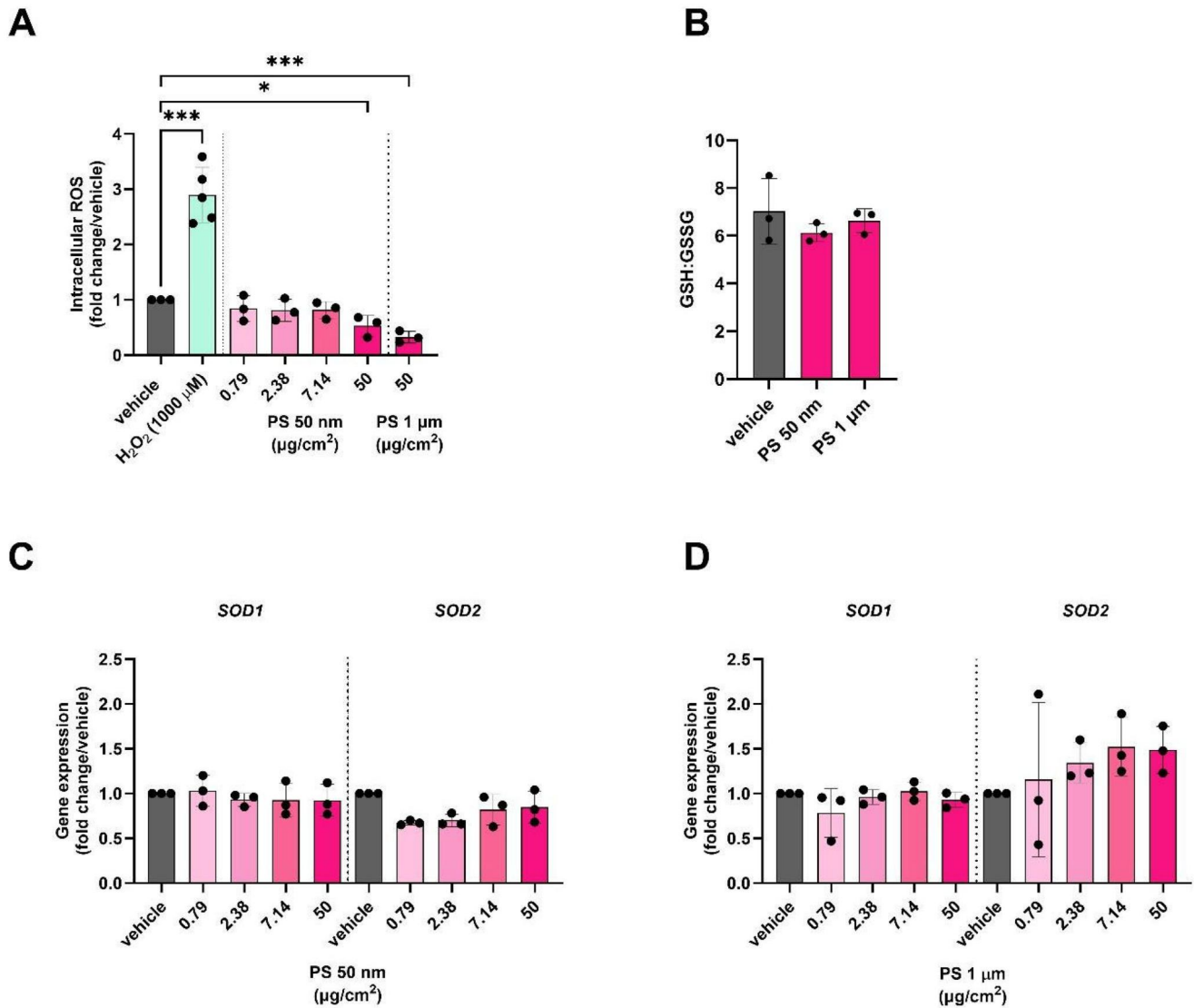


Figure 2. Intracellular ROS formation, GSH/GSSG ratio, and antioxidant gene expression of BEAS-2B cells in submerged culture upon exposure to polystyrene (PS) particles.

Cells were exposed to the vehicle control (exposure medium; DMEM, 0.05% BSA, 1% FBS) or PS particles for 24 h. Applied doses are displayed in $\mu\text{g}/\text{cm}^2$. IL-8 secretion (A). Gene expression of CXC Motif Chemokine Ligand 1 (*CXCL1*), CXC Motif Chemokine Ligand 2 (*CXCL2*), and CXC Motif Chemokine Ligand 8 (*CXCL8*) (B, C). Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) activation (D) and AP-1 activation (E) were measured through luminescence intensity in stably transfected BEAS-2B luciferase reporter cells. Data are shown as mean fold change over vehicle control $\pm$ SD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

glutathione disulfide (GSSG), as well as superoxide dismutase (*SOD1* and *SOD2*) transcript levels. We observed that neither exposure to PS 50 nm nor PS 1 μm induced changes in GSH/GSSG ratio (Figure 2(B)). In addition, no significant effects on antioxidant gene expression were observed after PS exposure (Figure 2(C,D)).

We next assessed whether PS exposure was able to induce inflammation in our bronchial epithelial cell model. While the positive control TNF- α potently induced IL-8 secretion (Figure S4(A)), neither 50 nm nor 1 μm PS particles led to significant IL-8 secretion in the medium after 24 h of exposure (Figure 3(A)). In

addition, TNF- α led to a massive induction of transcript levels of all 3 inflammatory genes we assessed (Figure S5(C)). Exposure to low doses of PS 50 nm particles slightly decreased *CXCL2* expression (FC = 0.63, $p = 0.006$ and FC = 0.71, $p = 0.02$) but did not lead to any significant changes in the expression of other inflammatory genes (Figure 3(B)). However, we did observe that the highest dose of PS microparticles, significantly induced mRNA expression of inflammatory genes *CXCL1* (FC = 4.47, $p = 0.02$), *CXCL2* (FC = 7.28, $p = 0.002$), and *CXCL8* (FC = 7.05, $p < 0.0001$). In addition, 2.38 $\mu\text{g}/\text{cm}^2$ PS microparticles also induced *CXCL1* gene expression (Figure 3(C)). In order to gain

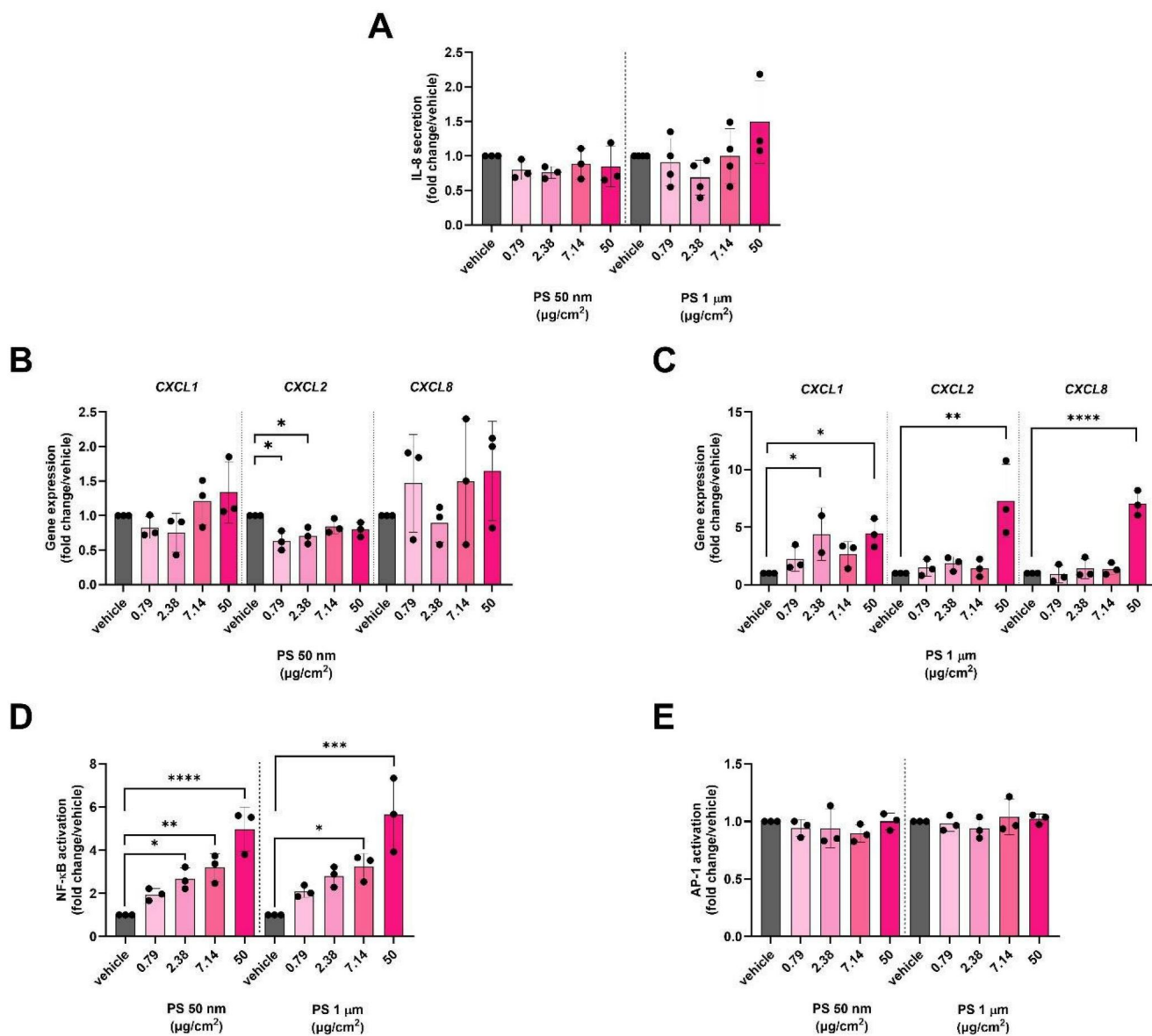


Figure 3. IL-8 secretion, pro-inflammatory gene expression and inflammatory pathway activation of BEAS-2B cells in submerged culture upon exposure to polystyrene (PS) particles.

Cells were exposed to the vehicle control (exposure medium; DMEM, 0.05% BSA, 1% FBS) or PS particles for 24 h. Applied doses are displayed in $\mu\text{g}/\text{cm}^2$. IL-8 secretion (A). Gene expression of CXC Motif Chemokine Ligand 1 (CXCL1), CXC Motif Chemokine Ligand 2 (CXCL2), and CXC Motif Chemokine Ligand 8 (CXCL8) (B, C). Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) activation (D) and AP-1 activation (E) were measured through luminescence intensity in stably transfected BEAS-2B luciferase reporter cells. Data are shown as mean fold change over vehicle control $\pm$ SD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

some mechanistic insight into which pathways were driving the enhanced expression of these genes in response to PS, we probed the transcriptional activity of two key inflammatory pathways known to drive expression of these genes, namely NF- κ B and AP-1 by use of specific BEAS-2B luciferase reporter cell lines. This revealed that TNF- α potentially increased NF- κ B transcriptional activity (FC = 28.0, $p = 0.02$), while AP-1 transcriptional activity was induced more moderately (FC = 1.44, $p = 0.002$) by TNF- α (Figure S5(D,E)). Subsequent exposure of these reporter cell lines to PS

50 nm and 1 μm particles showed that AP-1 transcriptional activity was unaltered while NF- κ B transcriptional activity was strongly upregulated in response to both sizes of PS particles in a dose-dependent manner (Figure 3(D,E)).

Effects of polystyrene particles on airlifted BEAS-2B cells

Evidently, exposure of BEAS to PS particles in a submerged condition is not fully representative of real-life

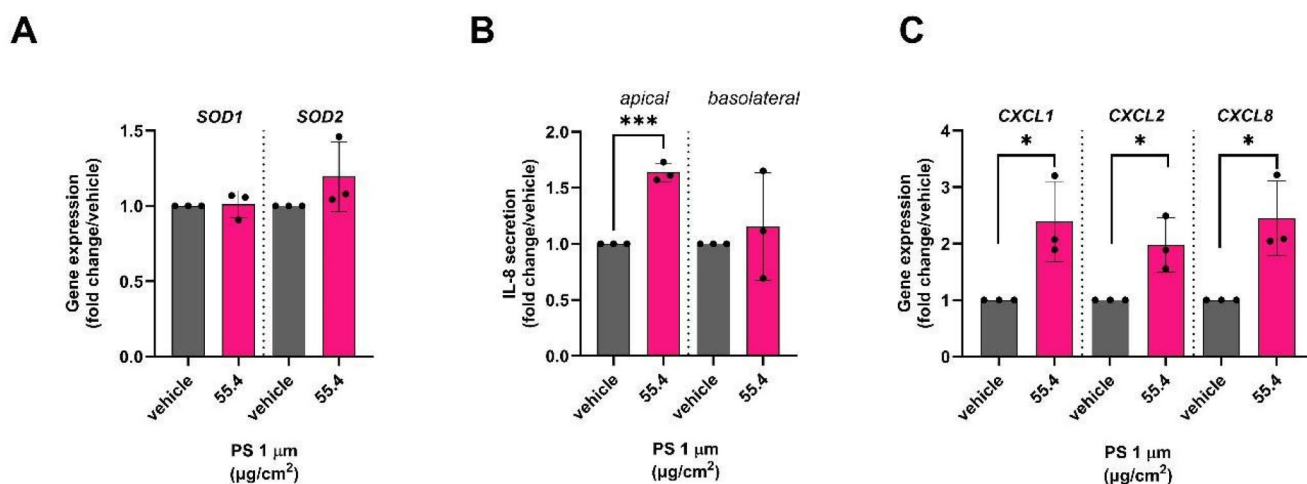


Figure 4. Effects of nebulized polystyrene (PS) microparticles on airlifted cultures of BEAS-2B cells.

Air-liquid interface (ALI) cultures of BEAS-2B cells were exposed to vehicle (MQ/PBS) or PS 1 μm particles at a concentration of 55.43 $\mu\text{g}/\text{cm}^2$ for 24 h. Gene expression of superoxide dismutase 1 (*SOD1*) and the mitochondrial manganese-dependent superoxide dismutase (*SOD2*) (A). IL-8 secretion upon exposure to 1 μm particles (B). Gene expression of CXC Motif Chemokine Ligand 1 (*CXCL1*), CXC Motif Chemokine Ligand 2 (*CXCL2*), and CXC Motif Chemokine Ligand 8 (*CXCL8*) (C). Data are shown as mean fold change over vehicle control $\pm$ SD. * $p < 0.05$; *** $p < 0.001$.

exposures *via* the inhalation route. Therefore, as a proof-of-concept, we next explored whether exposure of BEAS-2B cells cultured at the ALI to an aerosol of PS particles induced similar effects. Given that only the highest dose of PS particles, and most potently the 1 μm particles, had an impact on inflammatory gene expression, we selected this high dose (50 $\mu\text{g}/\text{cm}^2$) of 1 μm PS particles for ALI exposure experiments. Similar to submerged counterparts, assessment of LDH release in an apical wash of these cultures evidenced absence of cytotoxicity in response to PS exposure in this system (Figure S4(C)). As in observations in submerged cultures, PS 1 μm exposure of the ALI cultures did not result in any significant changes in antioxidant gene expression (Figure 4(A)). However, when assessing inflammatory status in response to PS 1 μm , we observed a significant increase in IL-8 secretion in the apical compartment (Figure 4(B)). In line with this, PS 1 μm exposure in ALI resulted in increases in *CXCL1*, *CXCL2*, and *CXCL8* gene expression, albeit smaller in magnitude compared to submerged cells (Figure 4(C)). Comparing the two exposure systems, the ALI cultures also appear to be less responsive to $\text{TNF-}\alpha$, in terms of IL-8 secretion, as well as *CXCL8* gene expression (Figure S5(A–D), ALI data adapted from Gosselink et al. (2024)). This might be explained by higher baseline inflammatory markers in ALI-cultured cells (Figure S2).

Discussion

In this study, we observed that PS toxicity in human BEAS-2B cells was size- and concentration-dependent

in the submerged system. Although both micro- and nanoparticles demonstrated low acute toxicity, high doses of 1- μm PS microparticles induced more pronounced proinflammatory effects than their smaller counterparts. In addition, with regard to dosimetry and cellular responses in conventional submerged cultures *vs.* aerosol exposures of the same cell line at ALI we observed that deposited dose and particle behavior were substantially different in both systems. This was associated with significant differences in the PS-induced inflammatory response, which was observed in both systems.

In our study, we assessed particle characteristics, deposition and cellular effects in submerged exposures *vs.* aerosol exposures. First of all, different exposure methods might require different dispersion protocols. While low zeta potential values were indicating strong electrostatic repulsion for PS particles in MQ, we were unable to measure zeta potential reliably in culture medium due to interference. The cell culture medium's high conductivity and FBS proteins adsorbing to particles is known to cause such interference (Bhattacharjee 2016). To prevent agglomeration and improve suspension stability, BSA is a commonly employed dispersant (Li et al. 2021). However, addition of (bio)surfactants might introduce artifacts and requires careful consideration (van Uunen et al. 2024). In submerged exposure medium, in presence of both FBS and BSA, we observed a substantial increase of the hydrodynamic diameter of PS nanoparticles, compared to PS nanoparticles dispersed in MQ. This effect, indicating adsorption of proteins from the medium onto the particle surface (protein corona), is known to affect

particle uptake and the availability of surface chemical groups (Leibe et al. 2019). Also, a corona of BSA has been demonstrated to reduce cytotoxic effects of PS nanoparticles in hepatocytes (80–200 nm) (Pan et al. 2026). Similar effects were observed for Copper (II) oxide nanoparticles, leading to less genotoxic effects in submerged BEAS-2B cells, in the presence of BSA (Siivola et al. 2020). Therefore, to make a better comparison, in future experiments BSA and FBS should also be included in the nebulized exposures. While ALI exposure did not contain FBS or BSA, it does require the presence of free ions and a salt solution is part of the standard operating procedure to assure accurate dosing (Ding et al. 2020). The co-deposition of salts, originating from the applied PBS/MQ, might have an impact on biological outcomes. To summarize, the different dispersion protocols of submerged exposure and ALI exposure lead to different exposure environments, complicating direct comparison between the two exposure modules.

In addition to interacting with medium components, MNPs might also interfere with readout assays (Kroll et al. 2012). This interference might account for the absence of IL-8 protein detection in our submerged exposures, despite evidence of induced inflammatory gene expression and NF- κ B activation. Whereas nebulization of particles avoids the use of FBS, the addition of a dispersion agent like BSA might still be necessary to create stable suspensions of MNPs with higher agglomeration tendency.

Since there is a discrepancy between the administered dose and delivered dose, accurate determination of the deposited dose is crucial to interpret toxicity outcomes. PS has a density of 1.05 g/cm³, which is higher than the exposure media density (0.98 g/cm³). However, particles might form agglomerates, with media fluid trapped between primary particles, lowering the total density of the agglomerate (Cohen et al. 2013). Indeed, Busch et al. (2021) measured a lowered PS effective density of 1.021 g/cm³ in MEM culture medium (1% FBS, density: 1.007 g/cm³). Their sedimentation modeling of 50 nm PS particles revealed a prediction of only 17% deposition after 24 h incubation (Busch et al. 2021). In addition, using the same methodology, we modeled deposition of the larger 1- μ m PS particles that we used and observed an even lower deposition efficiency of 8% after 24 h. Collectively, this convincingly shows that the deposited dose under submerged conditions in our study was most likely substantially lower than the dose deposited by nebulization. Dose measurements of ALI exposures with the integrated QCM allowed accurate

deposited dose determination, which is a clear advantage over submerged exposures. The high deposition efficiency in our ALI exposures demonstrates a complete and homogeneous nebulization process. However, taking into account that the cellular surface area only equals 7% of the total deposition area surface of the Vitrocell module (200.57 cm²), 5.3% of the applied dose reached the cells. Therefore, when low amounts of test substance are available, submerged exposure or small droplet application might still be the only option. In addition, for particle suspensions with higher agglomeration tendency, a lower deposition must be anticipated for, since part of the applied particles will remain clogged in the mesh of the vibrating membrane of the nebulizer (Motta et al. 2024).

Regarding cellular effects after PS exposure, we observed that neither PS nano- or microparticles caused cell death, increased oxidative stress levels or triggered activation of the antioxidant system. This is in line with another study, that did not observe cytotoxicity or increased ROS generation by PS 1 μ m (< 200 μ g/mL, corresponding with approximately 60–125 μ g/cm²) in submerged BEAS-2B cells, as measured with the DCFH assay (Jeon et al. 2023). It has to be mentioned that the decrease of fluorescence emission after treatment with the highest doses (50 μ g/cm²) of PS particles in our study might have resulted from quenching effects of the particles on the DCFH assay. Although widely used, the suitability of the DCFH probe to detect an intracellular oxidative stress response after (nano)particle toxicity has been questioned due to potential interference of the particles with the assay (Kalyanaraman et al. 2012; Ruijter et al. 2024). Ruijter et al. specifically identified interference when using the standard plate reader protocol, as they observed significantly higher fluorescence intensity when measuring DCF signal with flow cytometry. Further, when conducting another type of ROS detection method, a bioluminescent assay, others observed an increase of H₂O₂ in BEAS-2B cells after exposure to 10 μ g/cm² PS nanoparticles (200 nm) (Ernhofer et al. 2025). In contrast to our results and findings from the studies described above, another study observed increased cytotoxicity after exposing BEAS-2B cells to 10–30 μ g/cm² PS nanoparticles (40 nm) for 24 h (Yang et al. 2021). Discrepancies between *in vitro* studies using the same cell line and the same particle might result from variations in suspension preparation, exposure conditions or assay execution, indicating the importance of

standardization and proper particle characterization in exposure medium.

In this study, we observed size-dependent proinflammatory effects of PS in the submerged system, in which expression of pro-inflammatory genes potently increased after PS 1- μ m particle exposure and not after PS nanoparticles. This is in contrast to most previous studies, that often observe the opposite effect of size (Brown et al. 2001; Shi et al. 2022; Gosselink et al. 2025). One of the general hypotheses is that smaller sized particles exhibit higher uptake efficiency and therefore increased toxicity. Liu et al. indeed demonstrated a higher uptake of PS 50 nm particles by BEAS-2B cells compared to PS 100 nm particles for short time periods (< 12 h). However, after 24 h exposure the intracellular number of PS 100 nm particles was higher than PS 50 nm (Liu et al. 2022). In addition, the authors reported higher exocytosis for PS 50 nm compared to PS 100 nm. This highlights that, although we did not observe inflammatory gene upregulation after 24 h of exposure, there might be potential effects of PS nanoparticles at earlier time points. Another striking difference that we observed was that the inflammatory response differed in the submerged vs. the ALI system. While the submerged system displayed a more transcriptional inflammatory response, without increased IL-8 secretion, in the ALI system, the transcriptional response was much less pronounced while there was a significant increase in IL-8 secretion. The low deposition efficiency of the PS particles in the submerged system likely played a role in this difference. Taking this into account, the ALI system was exposed to a much higher dose, much faster than the submerged system which likely influences not only the magnitude of the inflammatory response but also the temporal profile.

Although this study provides valuable insights in the comparison of two important exposure methods for MNPs, some limitations have to be mentioned. Although PS is commonly used in the production of food packaging and biomedical devices, it only accounts for about 5% of the total European plastic production and is therefore not representative of the wide variety of MNPs in the environment (Plastics Europe 2024). Accurate and relevant dosing remains a challenge. *In vivo* exposure levels can currently only be estimated for larger-sized plastics due to analytical constraints. Such estimates for 24 h exposure have been much lower (152–507 microplastics/kg body weight per day) compared to our lowest dose (Eberhard et al. 2024). However, cumulative (lifetime) exposure should also be taken into account.

Moreover, in this study, we made use of a relatively simplistic cell culture model. The use of more complex *in vitro* models, including primary differentiated cultures and co-cultures, would make it easier to extrapolate results to real-live exposures. Given the nano size of the PS particles, it can be postulated that these would reach the alveolar region of the lungs, characterized by pneumocytes and macrophages. Testing the effect of PS nanoparticles in a co-culture of these two cell types would therefore be a relevant next step.

To conclude, this study highlights that there are substantial differences in aerosol exposures of MNPs vs submerged exposures. These include particle-medium interference (and thus particle behavior) and assessment of a precise effective dose. These differences likely influence biological responses. Even though submerged exposures might still function as a useful approach for high-throughput early-stage screening for MNP toxicity, ALI exposures will always have more biological relevance. Although promising, the field of MNP aerosol exposures is still developing and future investigations should include further optimization and validation of the use of aerosols for *in vitro* MNP risk analysis strategies.

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Authors' contributions

CRedit: **I. F. Gosselink**: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing; **P. Leonhardt**: Investigation, Visualization, Writing – original draft, Writing – review & editing; **M. J. Driittij**: Investigation; **E. M. Höppener**: Investigation; **R. J. Smelt**: Investigation; **F. J. van Schooten**: Project administration, Supervision; **A. H. V. Remels**: Conceptualization, Project administration, Supervision, Writing – review & editing. All authors read and approved the final manuscript.

Disclosure statement

The authors report there are no competing interests to declare.

Availability of data and materials

Data are publicly available on YODA, a data repository built and maintained by Utrecht University. Data can be found via the following link: <https://doi.org/10.24416/UU01-YYG5SC>. Data can be used under the Deed -

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References

- Allouche Y et al. 2025. Comparison of submerged and air liquid interface exposure: limitations and differences in the toxicological effects evaluated in bronchial epithelial cells. *Environ Res.* 279(Pt 2):121856. <https://doi.org/10.1016/j.envres.2025.121856>
- Amato-Lourenço LF et al. 2021. Presence of airborne microplastics in human lung tissue. *J Hazard Mater.* 416: 126124. <https://doi.org/10.1016/j.jhazmat.2021.126124>
- Bhattacharjee S. 2016. DLS and zeta potential—what they are and what they are not? *J Controlled Release.* 235: 337–351. <https://doi.org/10.1016/j.jconrel.2016.06.017>
- Braakhuis HM, Park MV, Gosens I, De Jong WH, Cassee FR. 2014. Physicochemical characteristics of nanomaterials that affect pulmonary inflammation. *Part Fibre Toxicol.* 11(1):18. <https://doi.org/10.1186/1743-8977-11-18>
- Brown DM, Wilson MR, MacNee W, Stone V, Donaldson K. 2001. Size-dependent proinflammatory effects of ultra-fine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicol Appl Pharmacol.* 175(3):191–199. <https://doi.org/10.1006/taap.2001.9240>
- Busch M, Bredeck G, Kämpfer AA, Schins RP. 2021. Investigations of acute effects of polystyrene and polyvinyl chloride micro- and nanoplastics in an advanced in vitro triple culture model of the healthy and inflamed intestine. *Environ Res.* 193:110536. <https://doi.org/10.1016/j.envres.2020.110536>
- Cohen J, DeLoid G, Pyrgiotakis G, Demokritou P. 2013. Interactions of engineered nanomaterials in physiological media and implications for in vitro dosimetry. *Nanotoxicology.* 7(4):417–431. <https://doi.org/10.3109/17435390.2012.666576>
- Danso IK, Woo J-H, Lee K. 2022. Pulmonary toxicity of polystyrene, polypropylene, and polyvinyl chloride microplastics in mice. *Molecules.* 27(22):7926. <https://doi.org/10.3390/molecules27227926>
- DeLoid GM et al. 2015. Advanced computational modeling for in vitro nanomaterial dosimetry. *Part Fibre Toxicol.* 12(1):32. <https://doi.org/10.1186/s12989-015-0109-1>
- DeLoid G et al. 2014. Estimating the effective density of engineered nanomaterials for in vitro dosimetry. *Nat Commun.* 5(1):3514. <https://doi.org/10.1038/ncomms4514>
- Ding Y et al. 2020. Quartz crystal microbalances (QCM) are suitable for real-time dosimetry in nanotoxicological studies using VITROCELL® Cloud cell exposure systems. *Part Fibre Toxicol.* 17(1):44. <https://doi.org/10.1186/s12989-020-00376-w>
- Dong CD et al. 2020. Polystyrene microplastic particles: in vitro pulmonary toxicity assessment. *J Hazard Mater.* 385:121575. <https://doi.org/10.1016/j.jhazmat.2019.121575>
- Eberhard T, Casillas G, Zarus GM, Barr DB. 2024. Systematic review of microplastics and nanoplastics in indoor and outdoor air: identifying a framework and data needs for quantifying human inhalation exposures. *J Expo Sci Environ Epidemiol.* 34(2):185–196. <https://doi.org/10.1038/s41370-023-00634-x>
- Ernhofer B et al. 2025. Small Particles, Big Problems: polystyrene nanoparticles induce DNA damage, oxidative stress, migration, and mitogenic pathways predominantly in non-malignant lung cells. *J Hazard Mater.* 495:139129. <https://doi.org/10.1016/j.jhazmat.2025.139129>
- Gosselink IF et al. 2024. Assessing toxicity of amorphous nanoplastics in airway- and lung epithelial cells using air-liquid interface models. *Chemosphere.* 368:143702. <https://doi.org/10.1016/j.chemosphere.2024.143702>
- Gosselink I et al. 2025. Size- and polymer-dependent toxicity of amorphous environmentally relevant micro- and nanoplastics in human bronchial epithelial cells. *Microplast Nanoplast.* 5(1):19. <https://doi.org/10.1186/s43591-025-00126-9>
- Halappanavar S, Mallach G. 2021. Adverse outcome pathways and in vitro toxicology strategies for microplastics hazard testing. *Curr Opin Toxicol.* 28:52–61. <https://doi.org/10.1016/j.cotox.2021.09.002>
- Housmans B et al. 2023. Direct comparison of non-osteoarthritic and osteoarthritic synovial fluid-induced intracellular chondrocyte signaling and phenotype changes. *Osteoarthritis Cartilage.* 31(1):60–71. <https://doi.org/10.1016/j.joca.2022.09.004>
- Hufnagel M, Schoch S, Wall J, Strauch BM, Hartwig A. 2020. Toxicity and gene expression profiling of copper- and titanium-based nanoparticles using air-liquid interface exposure. *Chem Res Toxicol.* 33(5):1237–1249. <https://doi.org/10.1021/acs.chemrestox.9b00489>
- Jenner LC et al. 2022. Detection of microplastics in human lung tissue using μ FTIR spectroscopy. *Sci Total Environ.* 831:154907. <https://doi.org/10.1016/j.scitotenv.2022.154907>
- Jeon MS et al. 2023. Polystyrene microplastic particles induce autophagic cell death in BEAS-2B human bronchial epithelial cells. *Environ Toxicol.* 38(2):359–367. <https://doi.org/10.1002/tox.23705>
- Kalyanaraman B et al. 2012. Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. *Free Radic Biol Med.* 52(1):1–6. <https://doi.org/10.1016/j.freeradbiomed.2011.09.030>
- Kaur K et al. 2022. Comparison of biological responses between submerged, pseudo-air-liquid interface, and air-liquid interface exposure of A549 and differentiated THP-1 co-cultures to combustion-derived particles. *J Environ Sci Health A Tox Hazard Subst Environ Eng.* 57(7):540–551. <https://doi.org/10.1080/10934529.2022.2083429>
- Kroll A, Pillukat MH, Hahn D, Schnekenburger J. 2012. Interference of engineered nanoparticles with in vitro toxicity assays. *Arch Toxicol.* 86(7):1123–1136. <https://doi.org/10.1007/s00204-012-0837-z>
- Leibe R et al. 2019. The protein corona suppresses the cytotoxic and pro-inflammatory response in lung epithelial cells and macrophages upon exposure to nanosilica. *Arch Toxicol.* 93(4):871–885. <https://doi.org/10.1007/s00204-019-02422-9>
- Li X, He E, Jiang K, Peijnenburg WJ, Qiu H. 2021. The crucial role of a protein corona in determining the aggregation kinetics and colloidal stability of polystyrene nanoplastics. *Water Res.* 190:116742. <https://doi.org/10.1016/j.watres.2020.116742>

- Liu YY et al. 2022. Endocytosis, distribution, and exocytosis of polystyrene nanoparticles in human lung cells. *Nanomaterials*. 13(1):84. <https://doi.org/10.3390/nano13010084>
- Luo WQ et al. 2025. Size-dependent internalization of polystyrene microplastics as a key factor in macrophages and systemic toxicity. *J Hazard Mater*. 490:137701. <https://doi.org/10.1016/j.jhazmat.2025.137701>
- Motta G et al. 2024. An integrated new approach methodology for inhalation risk assessment of safe and sustainable by design nanomaterials. *Environ Int*. 183:108420. <https://doi.org/10.1016/j.envint.2024.108420>
- Pan X, Yu X, Qin P, Yu W. 2026. Molecular mechanism underlying the modulated toxicity of differently charged and sized nanoplastics by bovine serum albumin. *J Photochem Photobiol B*. 274:113337. <https://doi.org/10.1016/j.jphotobiol.2025.113337>
- Plastics Europe. 2024. Plastics - the fast Facts 2024. Plastics Europe.
- Rahman I, Kode A, Biswas SK. 2006. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat Protoc*. 1(6):3159–3165. <https://doi.org/10.1038/nprot.2006.378>
- Ruijter N et al. 2024. Improving the dichloro-dihydro-fluorescein (DCFH) assay for the assessment of intracellular reactive oxygen species formation by nanomaterials. *NanoImpact*. 35:100521. <https://doi.org/10.1016/j.impact.2024.100521>
- Schmid O, Stoeger T. 2016. Surface area is the biologically most effective dose metric for acute nanoparticle toxicity in the lung. *J Aerosol Sci*. 99:133–143. <https://doi.org/10.1016/j.jaerosci.2015.12.006>
- Shi X et al. 2022. Cytotoxicity and genotoxicity of polystyrene micro-and nanoplastics with different size and surface modification in A549 cells. *Int J Nanomedicine*. 17:4509–4523. <https://doi.org/10.2147/IJN.S381776>
- Siivola KM, Suhonen S, Hartikainen M, Catalán J, Norppa H. 2020. Genotoxicity and cellular uptake of nanosized and fine copper oxide particles in human bronchial epithelial cells in vitro. *Mutat Res Genet Toxicol Environ Mutagen*. 856-857:503217. <https://doi.org/10.1016/j.mrgentox.2020.503217>
- Silva S, Bicker J, Falcão A, Fortuna A. 2023. Air-liquid interface (ALI) impact on different respiratory cell cultures. *Eur J Pharm Biopharm*. 184:62–82. <https://doi.org/10.1016/j.ejpb.2023.01.013>
- van Uunen D et al. 2024. Suspension of micro-and nanoplastic test materials: liquid compatibility,(bio) surfactants, toxicity and environmental relevance. *Environ Pollut*. 356:124306. <https://doi.org/10.1016/j.envpol.2024.124306>
- Yakovenko N et al. 2025. Human exposure to PM10 microplastics in indoor air. *PLoS One*. 20(7):e0328011. <https://doi.org/10.1371/journal.pone.0328011>
- Yang S et al. 2021. In vitro evaluation of nanoplastics using human lung epithelial cells, microarray analysis and co-culture model. *Ecotoxicol Environ Saf*. 226:112837. <https://doi.org/10.1016/j.ecoenv.2021.112837>
- Zhang X, Qiu J, Li X, Zhao J, Liu L. 2020. Complex refractive indices measurements of polymers in visible and near-infrared bands. *Appl Opt*. 59(8):2337–2344. <https://doi.org/10.1364/AO.383831>
- Zhang YX, Wang M, Yang L, Pan K, Miao AJ. 2022. Bioaccumulation of differently-sized polystyrene nanoplastics by human lung and intestine cells. *J Hazard Mater*. 439:129585. <https://doi.org/10.1016/j.jhazmat.2022.129585>
- Zhao X et al. 2023. Airborne microplastics: occurrence, sources, fate, risks and mitigation. *Sci Total Environ*. 858(Pt 2):159943. <https://doi.org/10.1016/j.scitotenv.2022.159943>