Evaluation of pulmonary effects of 3-D printer emissions from acrylonitrile butadiene styrene using an air-liquid interface model of primary normal human-derived bronchial epithelial cells_Dataset

GENERAL DESCRIPTION OF THE COLLECTION METHODS

1. Cell culture model

Primary NHBEs were purchased from PromoCell (cat. C-12640, PromoCell GmbH, Germany). Cryopreserved cells (healthy donor number 446Z036.9 and 432Z016.5; viability ≥ 94%; 500,000 cells per vial) were cultured and differentiated according to the manufacturer's instructions. Briefly, cells (passage P+1) were expanded in one T75 Corning™ Cell Culture Treated Flask, using the Pneumacult-Ex Plus expansion medium (cat. 05040, Stemcell Technologies, Vancouver, Canada), supplemented with 0.1% (v/v) hydrocortisone (cat. 07925, Stemcell Technologies). NHBE were cultured at 37 °C and under 5% CO_2 in air conditions, with a medium change every other day. After reaching 70% confluency, the cells were passaged into four T75 Corning™ Cell Culture Treated Flasks (cell density at 0.5 x 10^4 cells/cm^2) using an Animal Component-Free Cell Dissociation Kit (Stemcell Technologies) for 7 -10 min at 37 °C. The detached cells (passage P+2) were centrifuged at 350 g for 5 min and re-suspended in the expansion medium. Cells were cultured for additional 4-5 days in the expansion medium, with medium changed every other day, and subsequently used for the ALI procedure. To proceed with the ALI, 0.12 x 10^6 cells in 0.5 mL PneumaCult™-Ex Plus Medium were seeded on the apical side of inserts (0.4 µm pore membrane, Polyester, Costar® 12 mm Transwell®, Stemcell Technologies) in a 12-well plate with 1 mL expansion medium added into the basolateral chamber. The ALI condition was initiated 5 days post-seeding by the complete aspiration of the expansion medium in the apical compartment, thereby exposing the epithelial cells to the atmosphere (day 0 post-ALI) and replacing the medium from the basal compartment with the PneumaCult™-ALI Maintenance Medium (ALI medium). The ALI medium consisted of PneumaCult™-ALI Basal Medium containing 10% (v/v) PneumaCult™-ALI 10X Supplement, 1% (v/v) PneumaCult™-ALI Maintenance Supplement, 0.2% (v/v) heparin solution, 0.5% (v/v) hydrocortisone and 1% (v/v) penicillin/streptomycin. The cells were fed from the basal compartment with fresh ALI medium and maintained for 23 days before exposure to 3-D printer emissions.

2. Air-liquid interface cell exposure

2.1. Exposure system

The differentiated NHBEs cells were exposed to ABS 3-D printer emissions at the ALI using the same exposure system utilized previously to expose Sprague-Dawley rats

to emissions generated during real-time printing *via* a whole-body inhalation exposure system. The 3-D printer emissions generation chamber and exposure chamber were described in detail previously (Farcas et al., 2020).

2.2. Cell exposure treatment

Before the cell exposure treatment, cell morphology was inspected using phase-contrast microscopy followed by transepithelial electrical resistance (TEER) measurements, as described in the next paragraph.

Four different sets of treatments were employed as follows:

1) cell inserts were placed in the middle of the exposure chamber and exposed for 4 h to the ABS FFF 3-D printer emissions (marked as ABS-exposed cells),
2) another set of cells on inserts were kept inside the emissions chamber covered, with the lid on (marked as “exposure chamber” samples),
3) the third set of cells were maintained in the incubator at the normal growing conditions (marked as “incubator” samples), and
4) the last set of cells was treated on the apical surface of epithelial tissue with 0.1 ml 2% TX-100 (1 h prior to when the exposure ended) and used as a positive control for cytotoxicity and disruption of barrier integrity.

After the 4 h exposure treatment (time 0 h) and 24 h post-exposure, various endpoints were evaluated, such as changes in phenotypic characteristics (presence of ciliated, goblet, and basal cells), functional changes, cytotoxicity, and inflammatory and immune system regulation markers (cytokines, chemokines, and growth factors). To determine if the exposure system might be a source of contamination, a plate containing cell culture medium without antibiotics was kept open in the exposure chamber during the 4 h exposure. Next, the plate was maintained in the incubator for two weeks, inspected for contamination visually as well as by trypan blue stain. After two weeks, the media was clear and no contamination was observed, and no cells were present in the trypan blue-stained suspension.

3. Evaluation of epithelial barrier integrity

As an important indicator of barrier integrity, TEER was measured using an Evom2 Epithelial Volt/Ohm meter equipped with 4 mm chopstick electrodes (World Precision Instruments Inc., Sarasota, FL). TEER was measured prior to the treatment and at defined time points (0 and 24 h after the exposure). To measure TEER, 0.5 mL of the corresponding fresh medium was added to the apical side of a 12-well insert, equilibrated for an additional 30 min at 37 °C, 5% CO_2 in air, and then the chopstick electrodes were placed on either side of the primary airway epithelium. All TEER values were measured in duplicate for each well and corrected for cell-free insert (≈40 Ω) resistance and the surface area of a 12-well insert (1.12 cm^2).

4. Cytotoxicity

The possible cytotoxic effects of ABS emissions were assessed by measuring the cell viability and release of the cytosolic enzyme lactate dehydrogenase (LDH) into the medium.

4.1. Cell viability assay

AlamarBlue™ Cell Viability Reagent (cat. DAL1025, Thermo Fisher Scientific), a non-toxic, cell-permeable compound, was used to assess cell viability. The assay was performed using medium collected from the apical compartment. After harvesting culture supernatants from the basolateral chamber for further analysis (LDH and cytokines analysis), 200 µL of 10% AlamarBlue solution was added to each insert, and fresh medium was supplemented in the basolateral chamber. After 4 h incubation at 37 °C, the viability was measured by quantifying fluorescence levels (ex/em 560/590 nm) using the Synergy H1 hybrid multi-mode microplate reader (BioTek Instruments, Inc., Winooski, VT).

4.2. LDH release assay

The supernatant from the basal chamber was collected, and the release of the cytosolic enzyme LDH into the medium, indicative of cell membrane damage, was assayed spectrophotometrically by monitoring the reduction of nicotinamide adenine dinucleotide at 340 nm in the presence of lactate using a Lactate Dehydrogenase Reagent Set (Pointe Scientific, Inc., Lincoln Park, MI).

5. Measurement of cytokine release

Media collected from the bottom (basolateral) wells were stored at -80 °C until analysis. The concentrations of IL-17A, IL-21, IL-22, IL-23, IL-27, IL-31, MIP-3α, IL-17A/F, IL-17B, IL-17D, IL-3, IL-9, GM-CSF, IL-23p40, IL-15, IL-16, IL-17A, IL-1α, IL-5, IL-7, TNF-β, VEGF, IFN-γ, IL-10, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α, eotaxin, eotaxin-3, IL-8(HA), IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, and TARC (pg/ml) were measured using V-PLEX Human Biomarker Kits from Meso Scale Discovery (MSD, Meso Scale Discovery). Plates were read using MSD QuickPlex SQ 120 (Meso Scale Discovery) for electrochemiluminescence. Sample concentrations were derived from a standard curve plotted using a four-parameter logistic fit using MSD Workbench software.