Table S1. Primers used for quantitative real-time PCR (qPCR)

Gene name	Gene Symbol	Forward Primer 5'-3'	Reverse Primer 3'-5'
Beta (β)-Actin	ACTB	CTGGGACGACATGCAGAAAA	AAGGAAGGCTGGAAGAGTGC
Nuclear factor kappa-light-chain- enhancer of activated B cells	NFKB	AAGAGGAGGTTTCGCCACCG	TTGCAGATTTTGACCTGAGGGT
Tumor necrosis factor alpha	TNFα	AGCCCATGTTGTAGCAAACC	ACATTGGGTCCCCAGGATA
C-X-C Motif Chemokine Ligand 8	CXCL8	GCTCTGTGTGAAGGTGCAGTT	GGCACAGTGGAACAAGGACT
Interleukin 6	IL6	ACCCCCAGGAGAAGATTCCA	CACCAGGCAAGTCTCCTCATT
Superoxide Dismutase 3	SOD3	ACGCTGGCGAGGACGACCTG	GCTTCTTGCGCTCTGAGTGCTC
Glutathione S Transferase alpha 1	GSTA1	TGCAGCTGGAGTAGAGTTTG	ATGGGCACTTGCTGGAACAT
Glutathione Peroxidase1	GPX1	GTGCTCGGCTTCCCGTGCAAC	CTCGAAGAGCATGAAGTTGGGC
Heme oxygenase 1	HMOX1	TTCAAGCAGCTCTACCGCTC	GGGGGCAGAATCTTGCACTTT

Table S2. The checklist descriptors of the comparison between primary cells and 16HBE under airlifted culture conditions

	3D cell culture	Primary cells [7-9]	Cell line (16HBE)[10, 11]
	parameter		
Cells	Cell types	Ciliated cells, goblet cells,	Ciliated cells, goblet cells,
		Clara cells, basal cells	basal cells
	Cell availability	Limited	Unlimited
	Cell identity	Variance between different	Immortalized; standardized
		donors; donor's health affects	characterization
		model characteristics	
	Ethics	Ethical permission may be	Not required
		required	-
Cell culture	Handling	Require more experience; cells	Easier to work with
manipulation		sensitive to mistreatment	
	Culture medium	Serum-free medium; specific	Standardized medium
		growth factors; may need	
		optimization	
	Growth time	4 weeks before exposure	4 weeks (with extension
			possibility) before exposure
	Cost	High	Low
Biological	Quality standards	Differ between labs; usually	Standardized quality control
functions/features of		depends on experiment's	
culture		purpose	
	Culture	Pseudostratified structure; 3-5	Cobblestone morphology; 3-5
	morphology	layers of cells	cell layers

	Culture	Polarized mucociliary	Epithelial layer expresses
	functionality	differentiated airway epithelial	mucins and some cilia;
		cell layer	barrier function
	Relevance to	High; maintains morphology,	Low
	human tissue	gene expression, key metabolic	
		enzymes	
Assay validation for	Reproducibility of	Low (due to donor-to-donor	High
toxicity/efficacy	the results	variations)	
testing	Throughput	Low	High



Figure S1. Palladium nanoparticle (Pd-NP) induced cytotoxicity and apoptosis rate in Non-CB and CB mucosa models after 24h incubation following exposure to Pd-NP.

The cytotoxic effect was measured by colorimetric Lactate Dehydrogenase assay after 24 h of exposure to the clean air (sham) and all Pd-NP doses, positive Control (N=2) (A). The apoptosis rate was detected by FACS after 24 h of exposure to the clean air (sham) and high Pd-NP dose. Data presented as median and 25th -75th percentiles (N=3) (B). The gating strategy used to identify apoptotic cells (C).

References

- 1. Fothergill, S.J.R., D.F. Withers, and F.S. Clements, *Determination of Traces of Platinum and Palladium in the Atmosphere of a Platinum Refinery: By a Combined Chemical and Spectrographic Method.* British Journal of Industrial Medicine, 1945. **2**(2): p. 99-101.
- 2. Violante, N., et al., Assessment of workers' exposure to palladium in a catalyst production plant. J Environ Monit, 2005. **7**(5): p. 463-8.
- 3. *HTP-arvot 2014 Haitallisiksi tunnetut pitoisuudethttps*. 2014; Available from: <u>https://julkaisut.valtioneuvosto.fi/bitstream/handle/10024/162457/STM_2020_24_J.pdf</u>.
- 4. McCarrick, S., H.L. Karlsson, and U. Carlander, *Modelled lung deposition and retention of welding fume particles in occupational scenarios: a comparison to doses used in vitro.* Arch Toxicol, 2022. **96**(4): p. 969-985.
- 5. Pleil, J.D., M. Ariel Geer Wallace, M.D. Davis, and C.M. Matty, *The physics of human breathing: flow, timing, volume, and pressure parameters for normal, on-demand, and ventilator respiration.* J Breath Res, 2021. **15**(4).
- 6. Londahl, J., et al., *Measurement techniques for respiratory tract deposition of airborne nanoparticles: a critical review.* J Aerosol Med Pulm Drug Deliv, 2014. **27**(4): p. 229-54.
- 7. Ji, J., et al., *Development of Combining of Human Bronchial Mucosa Models with XposeALI(R)* for Exposure of Air Pollution Nanoparticles. PLoS One, 2017. **12**(1): p. e0170428.
- 8. Baxter, A., et al., *Targeted omics analyses, and metabolic enzyme activity assays demonstrate maintenance of key mucociliary characteristics in long term cultures of reconstituted human airway epithelia.* Toxicol In Vitro, 2015. **29**(5): p. 864-75.
- 9. Dvorak, A., et al., *Do airway epithelium air-liquid cultures represent the in vivo airway epithelium transcriptome?* Am J Respir Cell Mol Biol, 2011. **44**(4): p. 465-73.
- 10. Cozens, A.L., et al., *CFTR expression and chloride secretion in polarized immortal human bronchial epithelial cells*. Am J Respir Cell Mol Biol, 1994. **10**(1): p. 38-47.
- Forbes, B., A. Shah, G.P. Martin, and A.B. Lansley, *The human bronchial epithelial cell line* 16HBE14o- as a model system of the airways for studying drug transport. Int J Pharm, 2003.
 257(1-2): p. 161-7.