

Supplemental Material

Potential toxicity of micro- and nanoplastics in Primary Bronchial Epithelial Cells of patients with Chronic Obstructive Pulmonary Disease

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Table S1. Characteristics of Primary Bronchial Epithelial Cells donors

Donor	COPD stage	Sex (F/M)	Age (years)	Smoking status	Years quit before tissue collection	FEV₁/FVC ratio
1	Non-COPD	F	64	ES	Unknown	76.94
2	Non-COPD	M	73	ES	5	79.45
3	Non-COPD	M	72	ES	11	70.09
1	GOLD 2	M	58	ES	Unknown	53.59
2	GOLD 2	M	57	ES	3	61.14
3	GOLD 2	M	61	ES	Unknown	52.44

Abbreviations: COPD: chronic obstructive pulmonary disease, GOLD: global initiative for chronic obstructive lung disease, ES: ex-smoker, FEV₁: forced expiratory volume in the first second, FVC: forced vital capacity

Table S2. Primer sequences used for real time quantitative PCR analysis

Gene	Sense primer (5'→3')	Antisense primer (3'→5')
<i>ACTB</i>	AAGCCACCCCACTTCTCTCTAA	AATGCTATCACCTCCCCTGTGT
<i>B2M</i>	CTGTGCTCGCGCTACTCTCTCTT	TGAGTAAACCTGAATCTTTGGAGTACGC
<i>CYP4</i>	CATCTGCACTGCCAAGACTGA	TTCATGCCTTCTTTCACTTTGC
<i>RPL13A</i>	CCTGGAGGAGAAGAGGAAAGAGA	TTGAGGACCTCTGTGTATTTGTCAA
<i>CXCL8</i>	TTAGAACTATTAACAGCCAAACTCC ACA	CAAGTTTCAACCAGCAAGAAATTACTAAT ATTG
<i>MUC5AC</i>	AGCAGGGTCCTCATGAAGGTGGAT	AATGAGGACCCCAGACTGGCTGAA
<i>SCGB1A1</i>	TTCAGCGTGTTCATCGAAACCC	ACAGTGAGCTTTGGGCTATTTTT
<i>KRT5</i>	GGAGTTGGACCAGTCAACATC	TGGAGTAGTAGCTTCCACTGC
<i>TP63</i>	CCCGTTTCGTCAGAACACAC	CATAAGTCTCACGGCCCCCTC
<i>FOXJ1</i>	TCGTATGCCACGCTCATCTG	CTTGATAGATGGCCGACAGGG
<i>ZO-1</i>	AGGGGCAGTGGTGGTTTTCTGTTCTTTC	GCAGAGGTCAAAGTTCAAGGCTCAAGAGG
<i>OCN</i>	TCAGGGAATATCCACCTATCACTTCAG	CATCAGCAGCAGCCATGTACTCTTCAC
<i>CLDN3</i>	TTCATCGGCAGCAACATCATC	CGCCTGAAGGTCCTGTGG
<i>CLDN4</i>	ACAGACAAGCCTTACTCC	GGAAGAACAAGCAGAGAG
<i>SQSTM1</i>	GGTGCACCCCAATGTGATCT	CGCAGACGCTACACAAGTCG
<i>GABARAPL1</i>	ATCGGAAAAAGGAAGGAGAAAAGATC	CAGGCACCCTGGCTTTTGG
<i>SOD1</i>	GGTCCTCACTTTAATCCTCTAT	CATCTTTGTCAGCAGTCACATT
<i>SOD2</i>	TGGACAAACCTCAGCCCTAACG	TGATGGCTTCCAGCAACTCCC
<i>BAX</i>	GTCTTTTTCCGAGTGGCAGC	GTAGAAAAGGGCGACAACCC

Abbreviations: *ACTB*: beta-actin, *B2M*: beta-2 microglobulin, *CYP4*: Cyclophilin A, *Rpl13a*: ribosomal protein L13A, *CXCL8*: chemokine (C-X-C motif) ligand 8, *MUC5AC*: Mucin-5AC, *SCGB1a1*: Secretoglobulin Family 1A Member 1, *KRT5*: Keratin 5, *TP63*: tumor protein P63, *FOXJ1*: Forkhead box J1, *ZO-1*: zona occludens 1, *OCN*: occludin, *CLDN3*: Claudin 3, *CLDN4*: Claudin 4, *SQSTM1*: Sequestosome-1, *GABARAPL1*: GABA Type A Receptor Associated Protein Like 1, *SOD1*: Superoxide dismutase 1, *SOD2*: Superoxide dismutase 2, *BAX*: BCL2 associated X

Table S3. Antibodies and stains used for histochemistry

Target protein	Catalogue number	Company	Dilution
ACTB	T7451	Sigma	1:20,000
FoxJ1	14-9965-82	Invitrogen	1:200
Scgb1a1	HM2178	Sanbio	1:200
Rabbit-a-Mouse	E0413	DAKO	1:200
IgG/Biotin			
Swine-a-Rabbit	E0353	DAKO	1:200
IgG/Biotin			
Beta-actin	Ab176759	Abcam	1X (1:1000)
Nuclei acids	H3570	Thermo Fisher Scientific	1:2000

Abbreviations: ACT: Acetylated alpha-tubulin, FoxJ1: Forkhead box J1, Sgb1a1: Secretoglobin 1A1, IgG: Immunoglobulin G

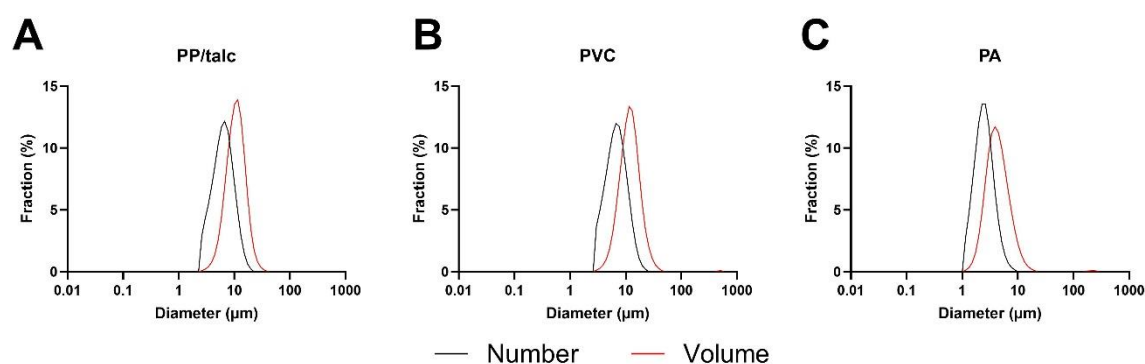


Figure S1 Size distribution spectra of micro- and nanoplastics Static light scattering (SLS) measurements of micro- and nanoplastics have been performed in PBS, 0.05% BSA, 1% 1-propanol). Number-based (black lines) and volume-based (red lines) distribution spectra are presented for polypropylene/talc (PP/Talc) (A), polyvinylchloride (PVC) (B) and polyamide-6 (PA) (C).

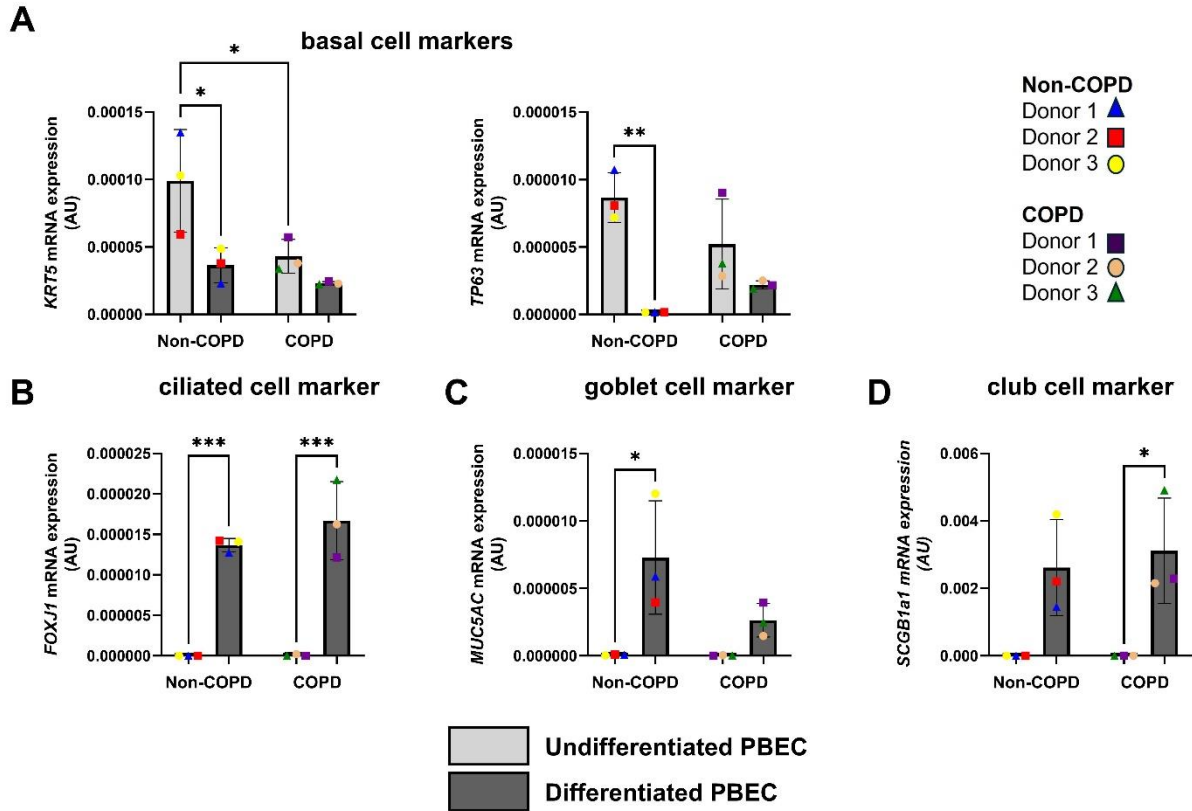


Figure S2 Characterization of differentiation status of primary bronchial epithelial cells (PBEC). mRNA expression of epithelial cell type-specific markers in undifferentiated and differentiated cultures. Basal cell markers: Keratin 5 (KRT5) and tumor protein P63 (TP63) (A). Ciliogenesis marker: Forkhead box J1 (FOXJ1) (B). Goblet cell marker: Mucin-5AC MUC5AC (C). Club cell secretory marker: Secretoglobin Family 1A Member 1 (SCGB1a1) (D). Each data point represents the mean of a technical triplicate of an individual donor. * $P < 0.05$, ** $P < 0.01$. ANOVA Bonferroni's multiple comparison's test.

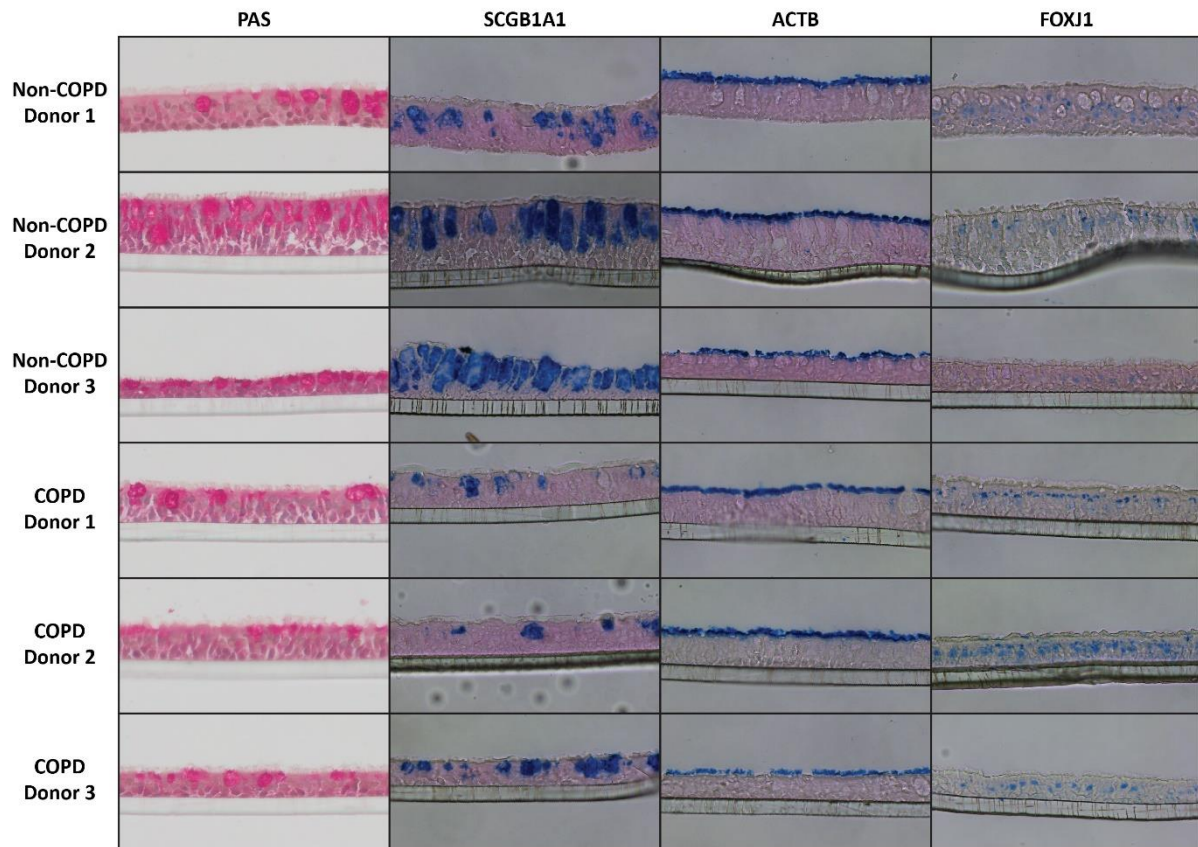


Figure S3 Immunohistochemistry staining of fully differentiated PBEC Periodic acid-Schiff (PAS; polysaccharides and mucosubstances), secretoglobin family 1A member 1 (SCGB1A1), cilia marker Acetylated Tubulin and Forkhead Box J1 (FOXJ1).

Table S4. Baseline differences between COPD and non-COPD PBEC

Feature	Non-COPD PBEC	COPD PBEC	Corresponding Figure
Cellular differentiation		Lower KRT5 mRNA expression in non-differentiated cultures (FC= 0.43, p=0.05)	Figure S2
Epithelial barrier morphology	2 donors present a pseudostratified columnar epithelium. Donor 3 epithelium is more flat (squamous)	Scgb1a1 protein levels lower for donors 1 and 2 (not quantified).	Figure S3
Epithelial barrier function	TEER increases to 345 (donor 1) and 290 (donor 2). For donor 3 TEER remains lower (197)	TEER increases to 200-245. Lower expression of tight junction proteins, especially <i>CLDN-4</i> (FC = 0.36, p= 0.02)	Figure 1
Inflammation	Baseline IL-8 secretion 3754-4539 pg/culture	Baseline IL-8 secretion 862-3789 pg/culture Higher IL-8 and <i>CXCL8</i> response to positive control (CuO)	Figure 3

Abbreviations: *KRT5*: Keratin 5, *Scgb1a1*: secretoglobin family 1A member 1, *TEER*: Trans-Epithelial Electrical Resistance, *IL-8*: Interleukin-8, *CXCL8*: chemokine (C-X-C motif) ligand 8, *CuO*: copper(II) oxide

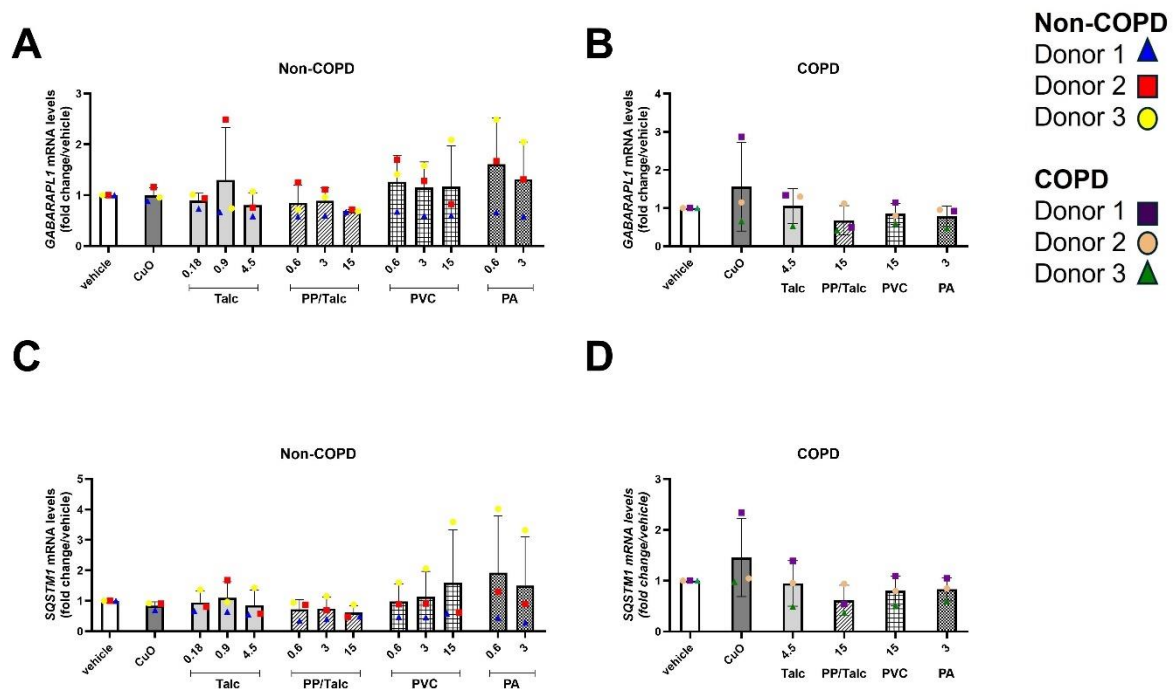


Figure S4 Effects of micro- and nanoplastics (MNPs), CuO and talc on key autophagy markers. Differentiated cultures of primary bronchial epithelial cells non-COPD (A) patients ($n=3$) and COPD (B) patients ($n=3$) were exposed to the vehicle control (dPBS, 0.05% BSA, 1% 1-propanol), $1.39 \mu\text{g}/\text{cm}^2$ copper(II) oxide (CuO), talc, polypropylene/talc (PP/Talc), polyvinylchloride (PVC) or polyamide-6 (PA) particles in dPBS, 0.05% BSA, 1% 1-propanol. Nominal doses are displayed on the X-axis, in $\mu\text{g}/\text{cm}^2$. GABA Type A Receptor Associated Protein Like 1 (GABARAPL1) (A,B) and Sequestosome-1 (SQSTM1) (C, D) mRNA levels as fold change compared to the vehicle control. For all experimental conditions, each data point represents the mean of a technical triplicate of an individual donor.

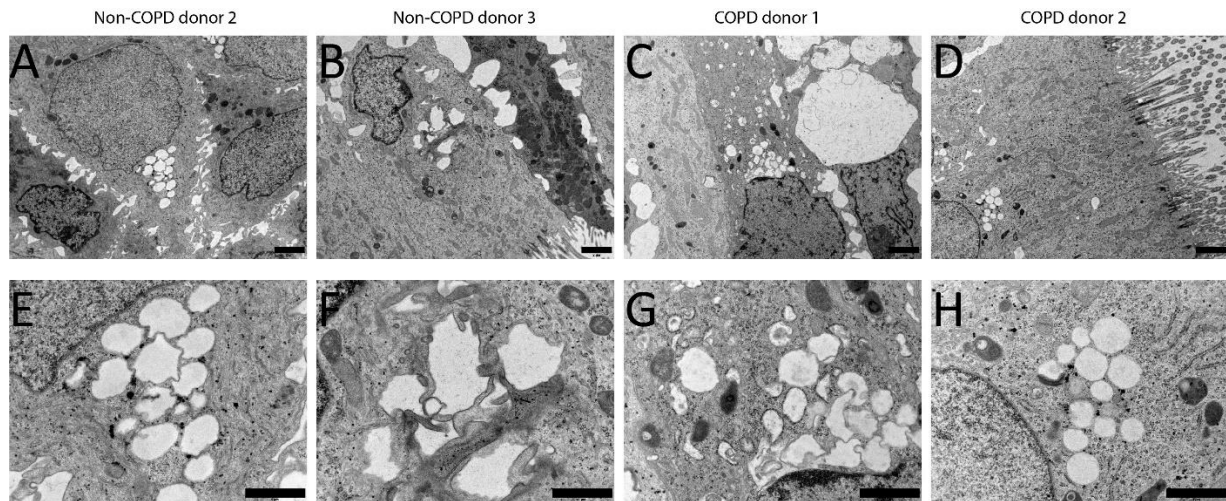


Figure S5 vesicle structures in non-COPD/COPD PBEC after polyamide nanoplastic exposure Transmission electron microscopy (TEM) micrographs of differentiated cultures of primary bronchial epithelial cells of non-COPD donor 2 (A, E), non-COPD donor 3 (B, F), COPD donor 1 (C, G) and COPD donor 2 (D, H), exposed to polyamide nanoplastics ($3 \mu\text{g}/\text{cm}^2$) for 24 h. The scale-bar in A-D represents $2 \mu\text{m}$. Micrographs E-H represent the same cultures, at higher magnification, with a scalebar of $1 \mu\text{m}$.