Supplementary material

Acrylonitrile Butadiene Styrene (ABS) and Polycarbonate (PC) Filament Three-Dimensional (3-D) Printer Emissions-Induced Cell Toxicity

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Figure S1. 3-D emissions collections setup. The FFF 3-D printer emissions were collected in a stainless-steel test chamber.

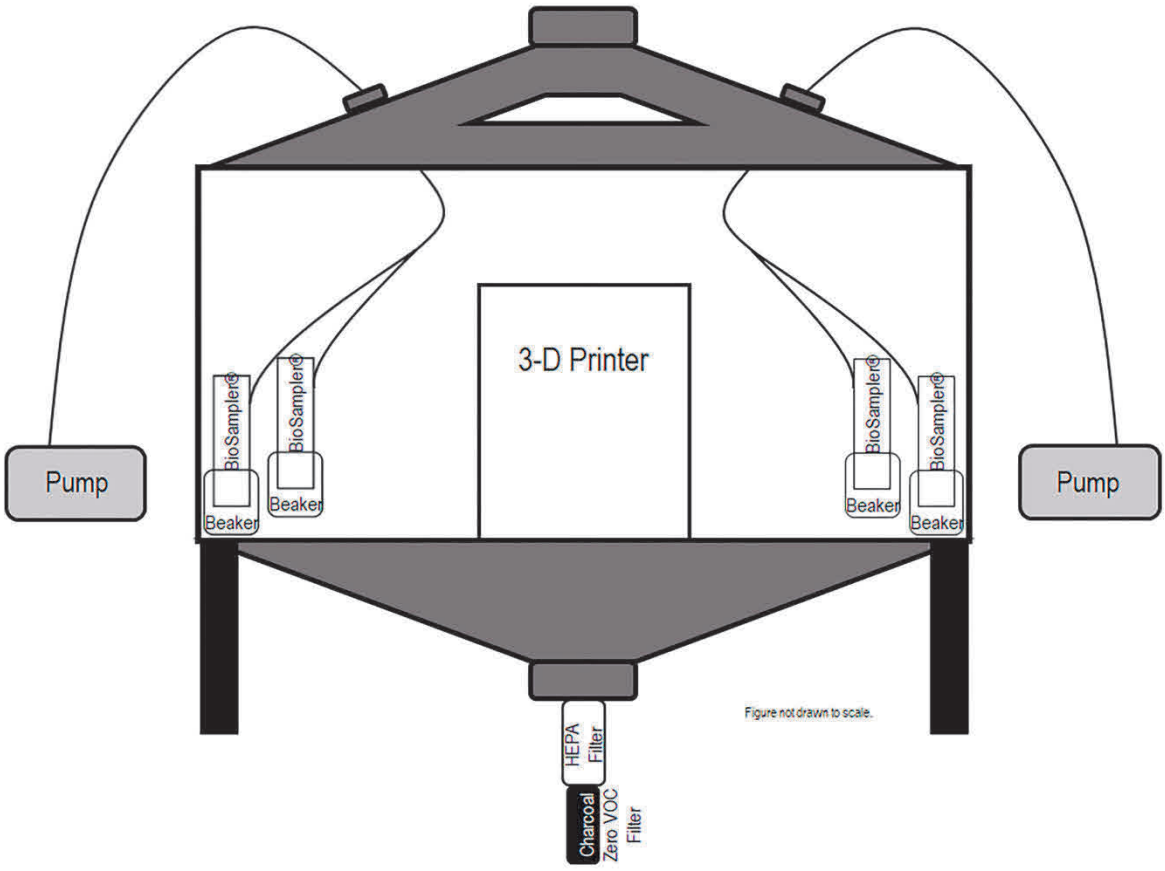


Table S1. Average particle concentrations per cm² of the background sample, PC and ABS collected emissions.

Sample type	Dilution	Average particles concentration (particles/ml)	Average particles/well	Average particles/cm²
Background	0%	2.67E+06	2.67E+05	8.34E+05
PC2	0%	3.47E+07	3.47E+06	1.08E+07
	25%	2.60E+07	2.60E+06	8.13E+06
	50%	1.74E+07	1.74E+06	5.42E+06
PC3	0%	6.24E+07	6.24E+06	1.95E+07
	25%	4.68E+07	4.68E+06	1.46E+07
	50%	3.12E+07	3.12E+06	9.75E+06
ABS2	0%	9.08E+06	9.08E+05	2.84E+06
	25%	6.81E+06	6.81E+05	2.13E+06
	50%	4.54E+06	4.54E+05	1.42E+06
ABS3	0%	1.51E+07	1.51E+06	4.72E+06
	25%	1.13E+07	1.13E+06	3.54E+06
	50%	7.55E+06	7.55E+05	2.36E+06

The particle concentrations were determined using nanoparticle tracking analysis (NTA) (NanoSight NS300, Malvern Instruments, Worcestershire, UK). SAEC were seeded in 96-well plates (surface area = 0.32 cm²) and exposed to 100 µL of PC or ABS particles/well collected in cell culture medium.

Calculation of estimated particles deposition fraction in the human alveoli based on the International Commission for Radiation Protection (ICRP) reference worker model for particles emitted during FFF 3-D printing with PC and ABS filament

For a print run using ABS that emits particles having geometric mean diameter of 22.7 nm (GSD = 1.3) modeling using MPPD2 (Asgharian et al., 2001) indicates that 35.8% of particles would deposit in the alveoli.

For PC, the geometric mean diameter of airborne particles released during printing is 47.5 nm (GSD = 1.3) and modeling indicates that 23.8% would deposit in the alveoli. Both particle sizes are means from n = 5 runs per filament from a prior study (Stefaniak et al., 2018).

Using default values for the International Commission for Radiation Protection (ICRP) reference worker model (20 breaths per minute, 1000 mL tidal volume, 3300 mL FRC volume) as a nose-mouth breather (ICRP. International Commission on Radiological Protection. Publication 66: Human respiratory tract model for radiological protection vol. 24. Pergamon: Oxford, UK, 1994):

ABS

8 hours work day = 480 min,

20 breaths /min,

1000 ml tidal volume,

- 480 x 20 x 1,000 = 9.6×10^6 total mL breath per work day

Assuming that the average particles concentration in the chamber = 2.5×10^5 particles/ cm^3 (reasonable based on our previous work (Yi et al., 2016), the total # of inhaled particles would be

- $5 \times 10^4 \times 9.6 \times 10^6 = 4.8 \times 10^{11}$ particles inhaled

Next, to get the # particles/ cm^2 of alveoli

- (total # inhaled particles) x (fraction deposited in alveoli region) / (total surface area of the lung) = $(4.8 \times 10^{11}) \times 0.358 / (102 \times 10^4) = 1.7 \times 10^5$ particles/ cm^2

PC

Same as above but using 0.238 as alveolar deposition fraction = 1.1×10^5 particles/ cm^2

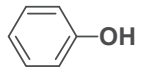
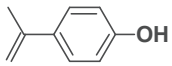
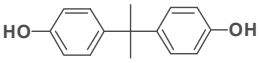
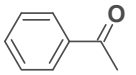
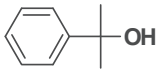
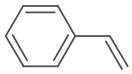
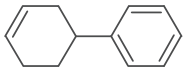
These calculated values are in good agreement with the in vitro dose values given in Table S2.

Table S2. Characterization of particles generated during a total of seven print runs (one background, three of PC, and three of ABS): concentration of undiluted collected samples and mean hydrodynamic diameter, mode diameter, and size of the particles in the 50th percentile were measured using NTA, and zeta potential was determined using ELS.

Sample	Concentration (particles/mL)	Mean Diameter (nm)	Mode Diameter (nm)	D50 (nm)	Zeta Potential (mV)
Background[a]	$0.267 \times 10^7 \pm 4.06 \times 10^5$	209.8 ± 13.1	183.9 ± 28.1	172.4 ± 12.6	-19.7 ± 0.7 (pH = 8.0)
PC1	$3.43 \times 10^7 \pm 1.17 \times 10^6$	232.6 ± 12.9	163.3 ± 13.0	201.6 ± 13.9	-15.7 ± 2.5 (pH = 7.9)
PC2	$3.47 \times 10^7 \pm 3.47 \times 10^6$	201.0 ± 8.0	151.5 ± 8.7	166.8 ± 7.3	-19.1 ± 0.8 (pH = 7.9)
PC3	$6.24 \times 10^7 \pm 3.66 \times 10^6$	169.5 ± 8.0	139.5 ± 4.2	147.2 ± 3.1	-19.4 ± 0.3 (pH = 7.9)
ABS1	$2.53 \times 10^7 \pm 1.22 \times 10^6$	217.3 ± 1.1	171.1 ± 15.9	196.0 ± 6.5	-21.3 ± 1.8 (pH = 7.8)
ABS2	$0.908 \times 10^7 \pm 1.39 \times 10^6$	192.5 ± 9.1	176.3 ± 34.7	173.6 ± 14.3	-17.6 ± 0.6 (pH = 8.0)
ABS3	$1.51 \times 10^7 \pm 1.47 \times 10^6$	197.5 ± 9.6	157.8 ± 27.3	170.0 ± 20.1	-18.0 ± 0.7 (pH = 8.0)

[a] "Background" refers to chamber air while the printer was powered off and not operating.

Table S3. Volatile organic compounds (VOCs) emitted during a total of seven print runs (one background, three of PC, and three of ABS) identified by GC-MS.

Sample	Retention Time (min)	Peak Area	Compound Name	Compound Structure	% Match
PC1	9.833	$3.04 \times 10^8 \pm 1.11 \times 10^7$	Phenol		94%
PC2		$4.65 \times 10^8 \pm 8.94 \times 10^7$			
PC3		$6.35 \times 10^8 \pm 6.41 \times 10^7$			
PC1	15.942	ND	p-Isopropenylphenol		94%
PC2		$5.04 \times 10^8 \pm 7.57 \times 10^7$			
PC3		$3.52 \times 10^8 \pm 3.13 \times 10^8$			
PC1	21.533	$0.568 \times 10^8 \pm 0.155 \times 10^8$	Bisphenol A		93%
PC2		$22.00 \times 10^8 \pm 1.42 \times 10^8$			
PC3		$13.50 \times 10^8 \pm 2.01 \times 10^8$			
ABS1	12.182	$0.302 \times 10^8 \pm 0.110 \times 10^8$	Acetophenone		96%
ABS2		$0.61 \times 10^8 \pm 6.42 \times 10^6$			
ABS3		$0.57 \times 10^8 \pm 1.03 \times 10^7$			
ABS1	12.797	$0.602 \times 10^8 \pm 0.717 \times 10^7$	α,α-Dimethylbenzenemethanol		94%
ABS2		$0.71 \times 10^8 \pm 6.30 \times 10^6$			
ABS3		$0.87 \times 10^8 \pm 2.57 \times 10^7$			
ABS1	17.103	$0.581 \times 10^8 \pm 1.88 \times 10^7$	Styrene		72%
ABS2		$0.85 \times 10^8 \pm 6.45 \times 10^6$			
ABS3		$1.10 \times 10^8 \pm 2.90 \times 10^7$			
ABS1	17.343	$0.652 \times 10^8 \pm 0.595 \times 10^7$	3-Cyclohexen-1-ylbenzene		78%
ABS2		$0.45 \times 10^8 \pm 3.70 \times 10^7$			
ABS3		$0.93 \times 10^8 \pm 2.51 \times 10^7$			

ND = not determined

Compounds were tentatively identified by comparing mass spectra to the 2014 NIST/EPA/NIH Mass Spectral Library (NIST 14); only compounds that matched ≥70% with the Library are reported herein.

Figure S2. Representative chromatograms of the organic compounds emitted during a total of seven print runs (one background, three of PC, and three of ABS). 3-D printer emissions were collected in cell culture medium and organic compounds were analyzed by SPME/GC-MS, followed by tentative identification by comparing mass spectra to the 2014 NIST/EPA/NIH Mass Spectral Library (NIST 14). As we did not quantify the levels of the chemicals, these data should be considered for information only.

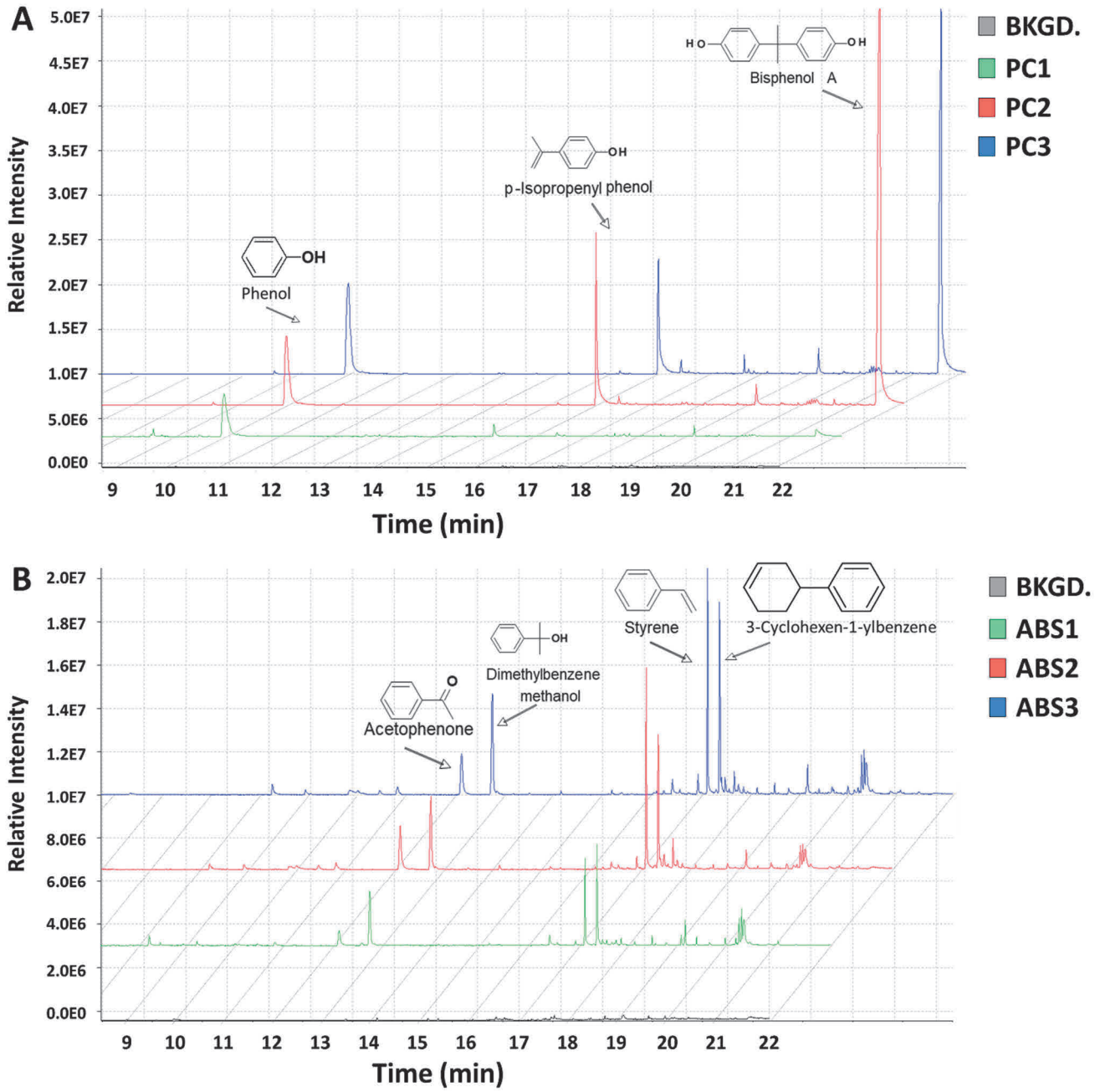
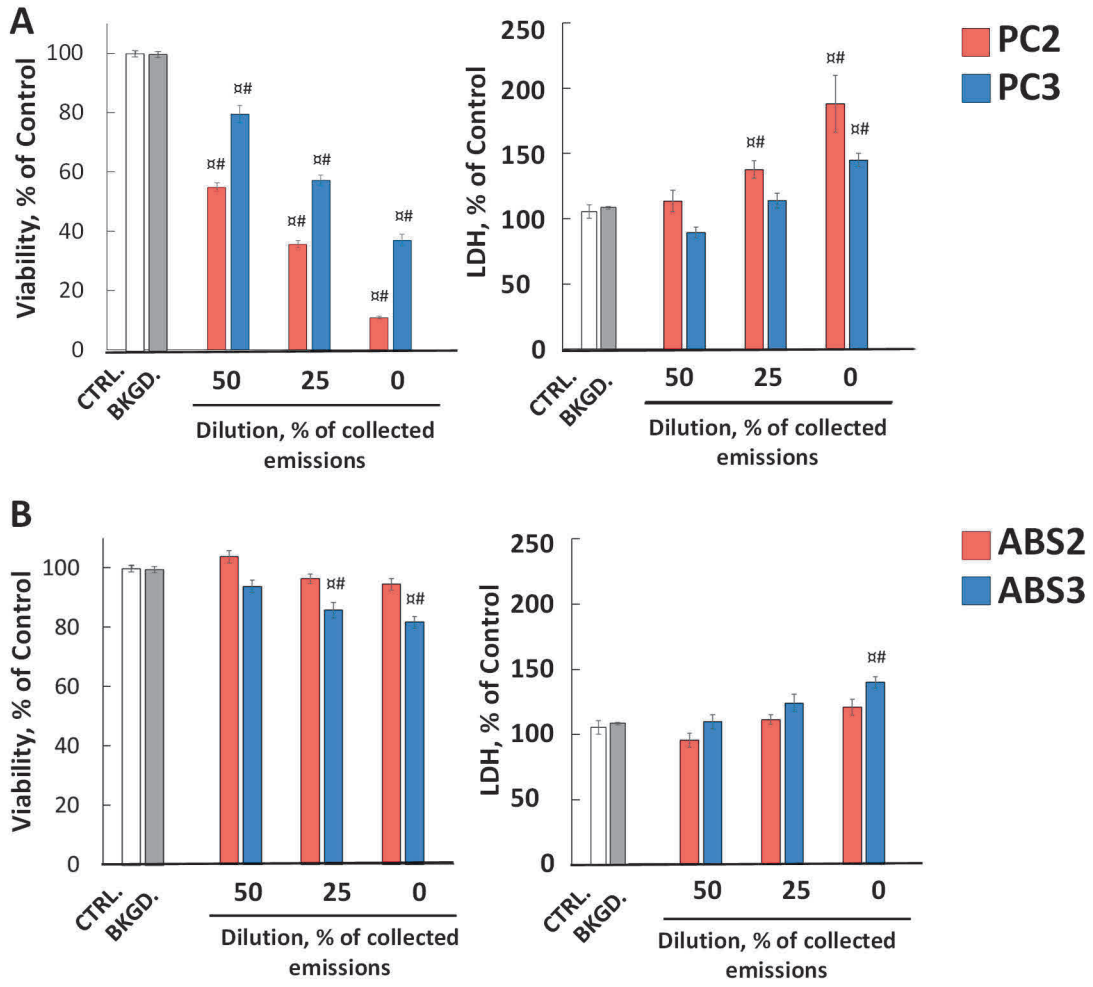


Figure S3. Cytotoxicity of the FFF 3-D printer emissions: cell viability and LDH activity of SAEC exposed to PC (A) and ABS (B) emissions. The PC (prints 2 and 3) and ABS (prints 2 and 3) collected emissions were subjected to vigorous vortex mixing for 5 min, and exposed as 50% dilution, and 25% dilution in serum-free SABM™, and undiluted (0%), resulting in six doses for each filament type (Supplemental Table S1). The background sample was used undiluted. The control samples were treated with SABM™.



Following one-way analyses of variance, where the assumptions of the model were examined, Dunnett's Test was used to determine significance of each treatment compared to either control non-treated cells or background ($p<0.05$). The symbol (¤) indicate statistically significant differences compared to serum-free cell culture medium treated control ($p < 0.05$). The number (#) signs indicate statistically significant differences compared to the background (ambient air) treated groups ($p < 0.05$).

Table S4. Cytokine and chemokine production in SAECs exposed to FFF 3-D printer emissions. SAEC were exposed the PC and ABS emissions in culture medium for 24 h. The PC (print 2 and 3) and ABS (print 2 and 3) collected emissions were subjected to vigorous vortex mixing for 5 min, and exposed as 50% dilution, and 25% dilution in serum-free SABM™, and undiluted (0%), resulting in six doses for each filament type (Supplemental Table S1). The background sample was used undiluted. The control samples were treated with SABM™. Experiments were performed in three independent experiments with n = 3 replicates each.

Agent	Dilution, %	Average particles/cm²	Analyte, pg/total cells (x 10^-4) ± SEM							
			IL-12p70	IL-1α	IL-13	IL-1β	IL-6	IL-8	IL-16	TNF-α
Control	NA	0	1.427 ± 0.48	1.287 ± 0.01	696.209 ± 24.68	0.791 ± 0.04	145.978 ± 2.77	4265.423 ± 132.75	21.603 ± 4.69	2.281 ± 0.12
BKGD.	0	0.834E+06	1.935 ± 0.21	1.902 ± 0.24	887.601 ± 88.93	0.954 ± 0.07	129.15 ± 34.98	5318.803 ± 159.51	32.795 ± 3.62	2.087 ± 0.07
PC2	50	5.42E+06	2.704 ± 0.07	4.310 ± 0.47	1431.56 ± 72.08*#	1.572 ± 0.06	927.116 ± 37*#	11082.741 ± 184.21*#	65.197 ± 8.11	4.112 ± 0.04
	25	8.13E+06	3.980 ± 0.59*#	8.117 ± 0.3*#	2418.064 ± 244.37*#	2.761 ± 0.15	784.944 ± 23.43*#	20559.863 ± 272.47*#	110.102 ± 11.65*#	5.912 ± 0.37*#
	0	10.8E+06	13.171 ± 0.98*#	62.670 ± 3.15*#	6278.268 ± 249.32*#	370.918 ± 48.43*#	976.032 ± 17.63*#	32377.009 ± 842.63*#	371.096 ± 47.47*#	17.907 ± 1.56*#
PC3	50	9.75E+06	1.445 ± 0.47	1.260 ± 0.07	786.841 ± 113.71	1.105 ± 0.03	255.645 ± 15.79*#	3388.327 ± 515.7	27.951 ± 13.79	1.967 ± 0.23
	25	14.6E+06	2.550 ± 0.44	2.098 ± 0.36	1281.809 ± 47.44*#	1.424 ± 0.26	367.843 ± 6.19*#	8654.719 ± 224.36*#	48.326 ± 6.42	3.875 ± 0.23
	0	19.5E+06	3.039 ± 0.22*#	3.261 ± 0.12	1736.912 ± 94.12*#	2.352 ± 0.08	259.588 ± 3.81*#	9050.036 ± 40.02*#	106.033 ± 12.12*#	6.154 ± 0.18*#
ABS2	50	1.42E+06	1.263 ± 0.24	0.848 ± 0.01	778.315 ± 58.09	0.985 ± 0.03	186.598 ± 7.16	6529.206 ± 56.22*#	40.247 ± 5.58	2.288 ± 0.29
	25	2.13E+06)	1.377 ± 0.16	1.004 ± 0.06	610.418 ± 48.05	0.763 ± 0.07	193.677 ± 1.59	4720.557 ± 104.95	31.410 ± 1.03	1.793 ± 0.11
	0	2.84E+06	1.135 ± 0.07	1.126 ± 0.09	674.216 ± 27.35	0.751 ± 0.02	234.538 ± 8.1*#	5424.276 ± 73.44	30.753 ± 2.41	1.868 ± 0.03
ABS3	50	2.36E+06	1.886 ± 0.34	1.483 ± 0.24	603.601 ± 26.87	0.928 ± 0.04	131.905 ± 6.15	3925.374 ± 63.63	29.871 ± 0.22	1.989 ± 0.11
	25	3.54E+06	1.642 ± 0.35	1.275 ± 0.01	672.43 ± 34.94	1.329 ± 0.1	135.475 ± 1.18	2468.865 ± 40.22*#	38.818 ± 0.02	1.842 ± 0.18
	0	4.72E+06	3.228 ± 0.68	2.490 ± 0.32	1133.304 ± 260.87	1.386 ± 0.01	208.989 ± 5.67*#	5039.533 ± 99.95	67.015 ± 2.54*#	2.414 ± 0.31

Following one-way analyses of variance, where the assumptions of the model were examined, Dunnett's Test was used to determine significance of each treatment compared to either control or background ($p<0.05$). The symbol (¤) indicate statistically significant differences compared to serum-free cell culture medium treated control ($p < 0.05$). The number (#) signs indicate statistically significant differences compared to the background (ambient air) treated groups ($p < 0.05$).

References

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