Supplementary information

Design of a Multilayer Lung Chip with Multigenerational Alveolar Ducts to Investigate the Inhaled Particle Deposition

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Video S1: Continuous photography of the flow field for one respiratory cycle.

Method for measuring the particle size distribution: The diameter of the particles was measured with an inverted fluorescence microscope (IX73; Olympus, Tokyo, Japan). Sodium fluorescein (molecular weight = 376.27 g/mol) solution in water was sprayed at a rate of 2 g/L. The nebulizer was turned on and the applicator was aimed vertically at a glass slide with a side length of 5 cm. The spraying time is approximately 2 s. Immediately after, the slides were placed on the microscope for photography. The obtained photographs were post-processed using ImageJ software.

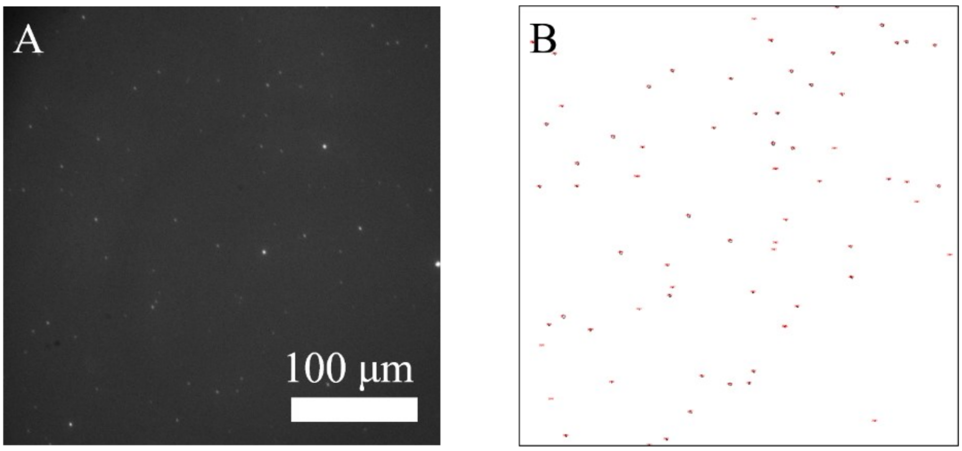

Fig. S1 Photographs of individual particle positions on a glass piece. (A) Snapshots of a glass piece with a 2 s spray time. (B) Results of post-processing using ImageJ software.

Due to the limited resolution of the microscope, particles with diameters less than 0.2 μm are ignored. The particle data were found to be statistically close to a normal distribution with a count mean diameter of 1.32 μm. The particle size distribution data were used to set the particle diameter distribution in the numerical simulation.

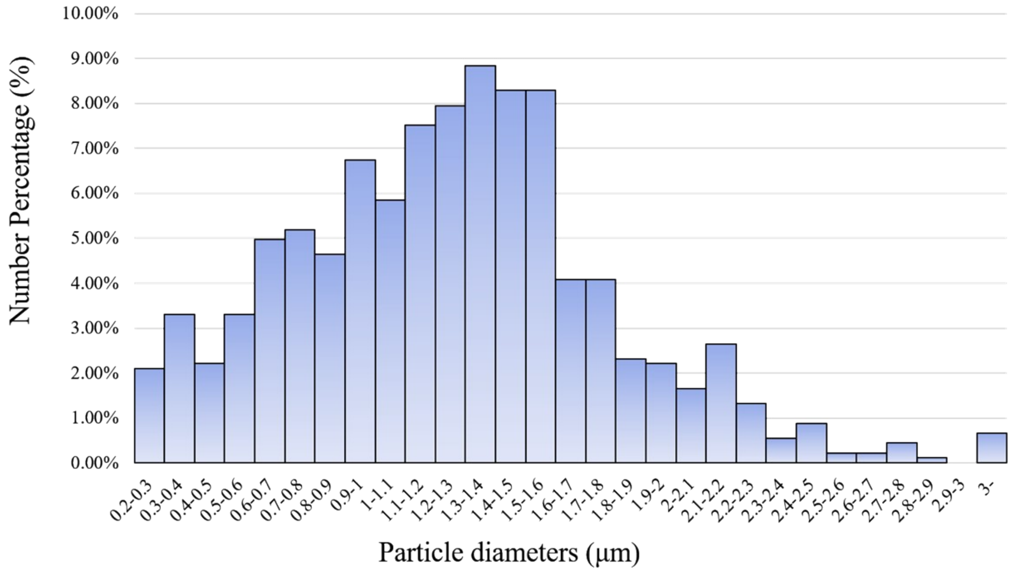

Fig. S2 Diameter distribution statistics of aerosols emitted from the sprayer.

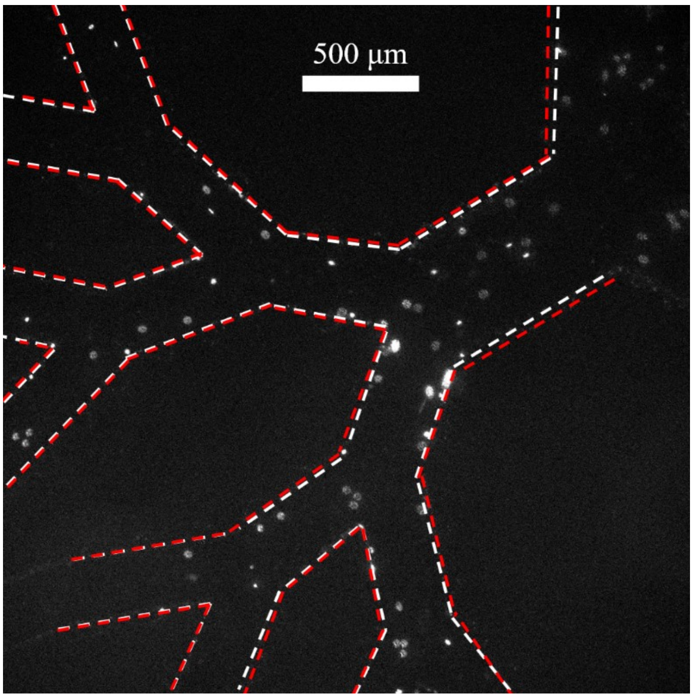

Fig. S3 The expansion of the PDMS ducts during the respiration cycle. The white dashed line represents the position of the wall at t=0, at which point all the air is exhaled and the wall displacement along its normal direction is minimal; the red dashed line corresponds to the position of the wall at t=0.5T, at which point the inhalation volume is at its maximum, and the wall displacement along its normal direction is at its maximum.

Calculation of flow rates in an ideal multi-generational tree-bifurcated tracheal structure: Assuming that each generation of trachea is divided into two equal next-generation tracheae, we obtain the flow rate of each generation of alveolar ducts as

$$Q_{d,i+1} = \frac{Q_{d,i}}{2} \quad \text{(S1)}$$

where i = 0, 1, 2, 3, …, and 23. For an adult weighing 50 kg, whose lungs inhale a tidal volume of airflow of approximately 500 mL within an inspiration time of 2 s, the average flow rate in the 16th generation airways can be calculated from equation (S1) as 3.81 μL/s. The polytetrafluoroethylene tube used in the experiment has a diameter of 0.5 mm, and the corresponding inspiratory flow velocity can be calculated to be 19.43 mm/s.

Table. S1 Standard deviation of particle deposition rates for different respiration conditions after two breathing cycles

Generation	1 (16th)	2 (17th)	3 (18th)	4 (19th)	5 (20th)
a	0.069	0.023	0.044	0.036	0.059
b	0.066	0.056	0.032	0.065	0.060
c	0.065	0.043	0.003	0.004	0.101
d	0.045	0.061	0.044	0.024	0.061
e	0.064	0.054	0.036	0.015	0.091

Table. S2 Standard deviation of particle deposition rates for different respiration conditions after five breathing cycles

Generation	1 (16th)	2 (17th)	3 (18th)	4 (19th)	5 (20th)
a	0.040	0.025	0.010	0.016	0.063
b	0.031	0.018	0.019	0.025	0.035
c	0.106	0.081	0.080	0.006	0.114
d	0.020	0.002	0.032	0.002	0.053
e	0.044	0.056	0.025	0.019	0.049

Table. S3 P-values of particle deposition rates for different respiration conditions for two and five respiratory cycles

Generation	1 (16th)	2 (17th)	3 (18th)	4 (19th)	5 (20th)
a	0.968	0.327	0.191	0.129	0.755
b	0.375	0.590	0.918	0.556	0.447
c	0.808	0.773	0.680	0.022	0.999
d	0.308	0.667	0.877	0.022	0.919
e	0.750	0.839	0.401	0.807	0.937