UK Health Security Agency

NIHR | Health Protection Research Unit in Environmental Exposures and Health at Imperial College London

Validation of an Aerosol Exposure Air-Liquid-Interface (AE-ALI) system to facilitate more realistic hazard identification of nano-sized aerosol exposure in human relevant culture models

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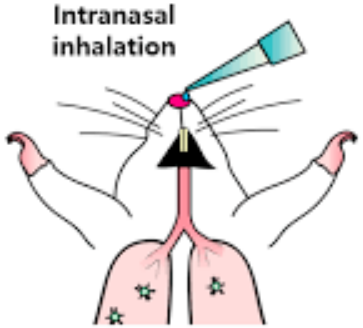
Background

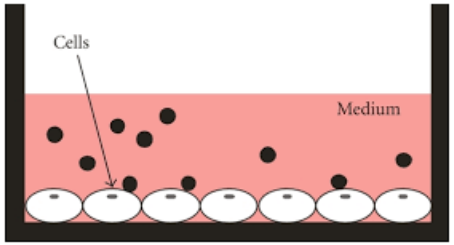
- Air pollution is one of the greatest environmental risk to public health, for which ambient particulate matter (PM) is considered the major contributor.
- The increasing use of the engineered nanomaterials (ENMs) also raised concerns over inadvertent exposure and the potential for hazardous effects on exposure in humans through the inhalation route.
- There is emerging need to identify specific inhalation hazards by utilising appropriate exposure models.

Exposure models

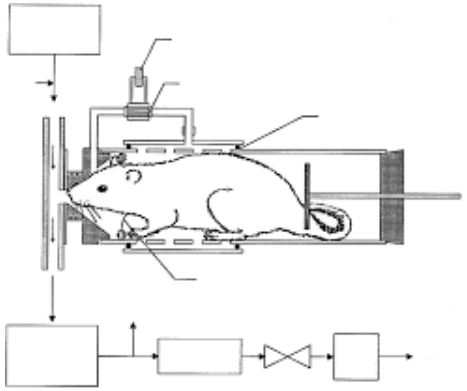
- Rodent models



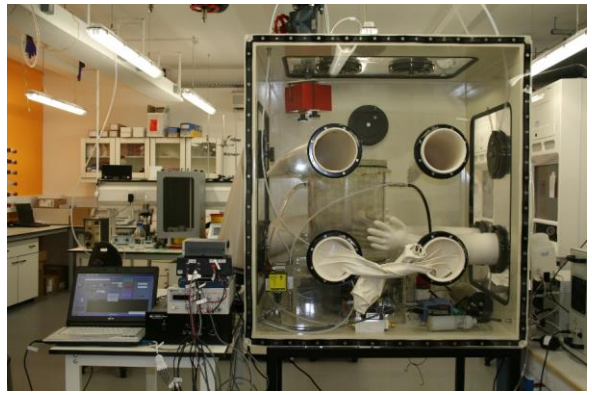
- Submerged cultured conditions



Nose-only inhalation

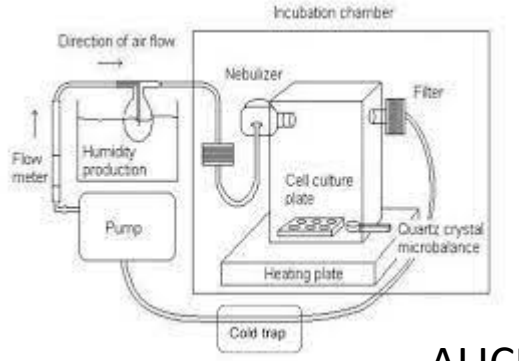


Whole-body exposure



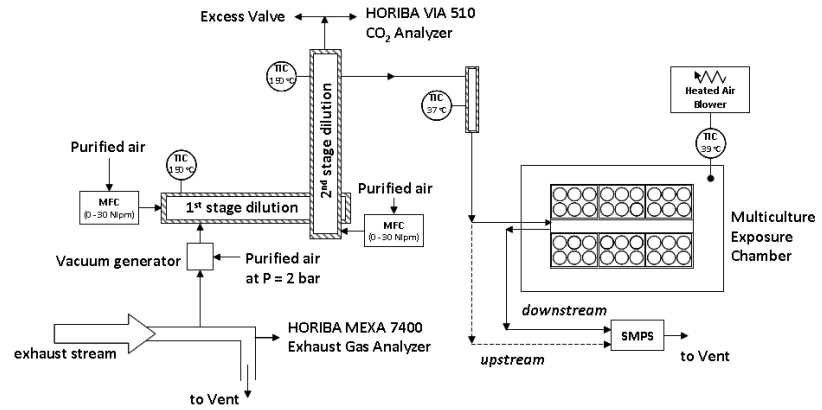
Air-liquid interface (ALI) exposure

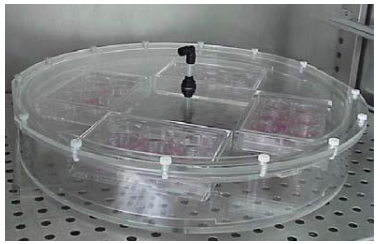
- Better to mimic *in vivo* inhalation experiments of airborne particles



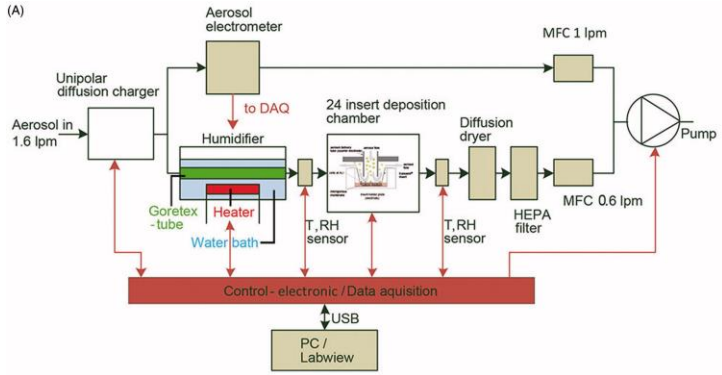
ALICE

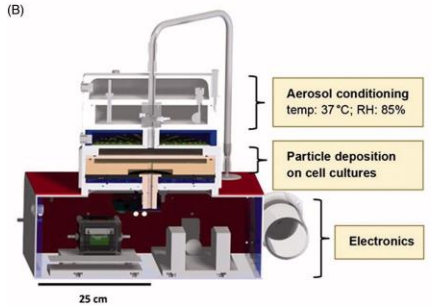
CLOUD©

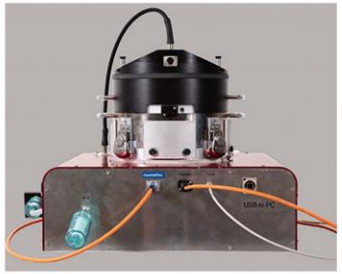




MEC

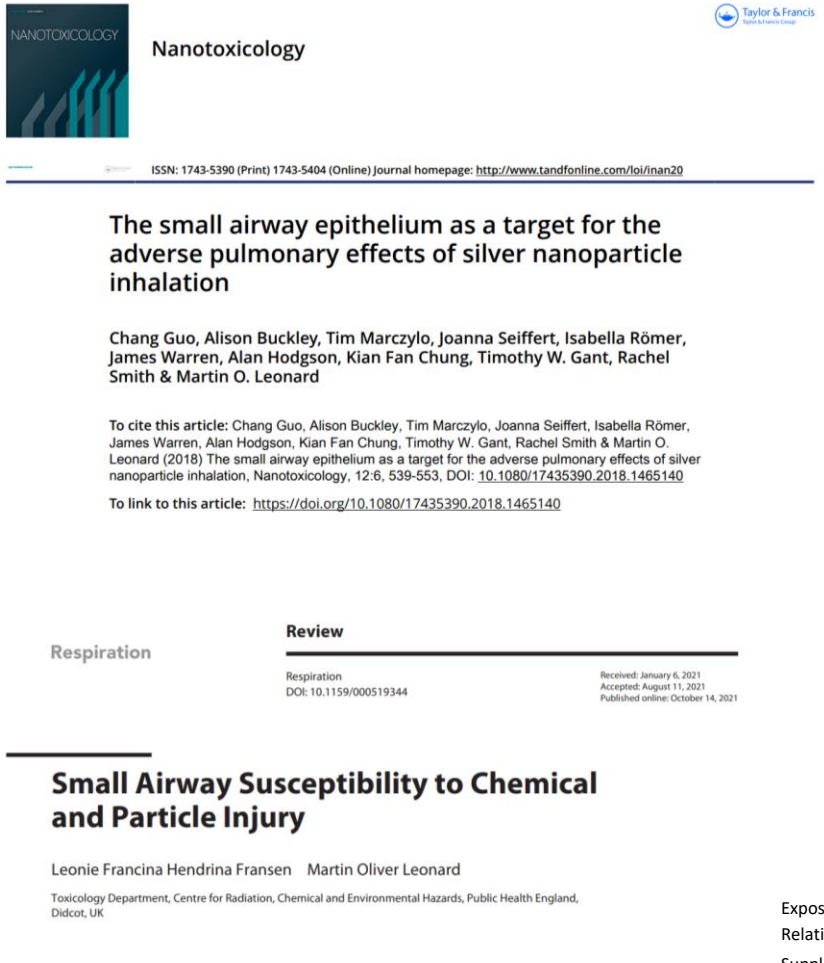






NACIVT

Previous *in vitro* inhalation studies on ENMs



Nanotoxicology

Taylor & Francis

ISSN: 1743-5390 (Print) 1743-5404 (Online) Journal homepage: http://www.tandfonline.com/loi/inan20

The small airway epithelium as a target for the adverse pulmonary effects of silver nanoparticle inhalation

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To cite this article: Chang Guo, Alison Buckley, Tim Marczylo, Joanna Seiffert, Isabella Römer, James Warren, Alan Hodgson, Kian Fan Chung, Timothy W. Gant, Rachel Smith & Martin O. Leonard (2018) The small airway epithelium as a target for the adverse pulmonary effects of silver nanoparticle inhalation, Nanotoxicology, 12:6, 539-553, DOI: 10.1080/17435390.2018.1465140

To link to this article: https://doi.org/10.1080/17435390.2018.1465140

Respiration

Review

Respiration
DOI: 10.1159/000519344

Received: January 6, 2021
Accepted: August 11, 2021
Published online: October 14, 2021

Small Airway Susceptibility to Chemical and Particle Injury

Leonie Francina Hendrina Fransen Martin Oliver Leonard

Toxicology Department, Centre for Radiation, Chemical and Environmental Hazards, Public Health England, Didcot, UK

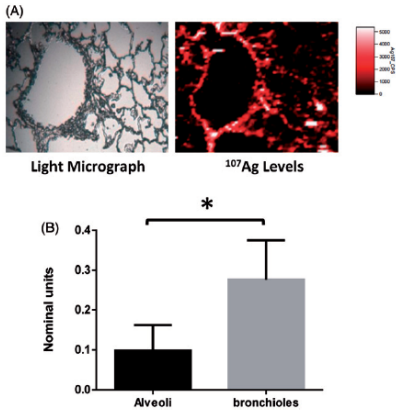
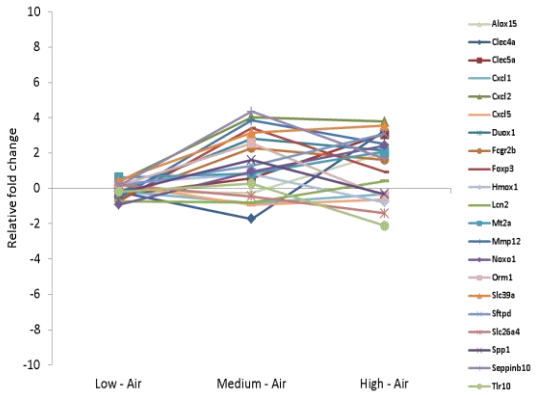


Figure 4. Analysis of ^{107}Ag levels with rat lung after AgNP exposure. After AgNP exposure, lung tissue was fixed and processed for laser ablation ICP-MS assessment of ^{107}Ag levels.



Exposure duration (min)	7	20	60	
Relative to AgNP deposition	1.5	4.4	13.2	ng/cm^2

Supplementary Figure Sensitivity of a set of Stress Response genes to air exposure in SmallAIR cultures in AE-ALI system.

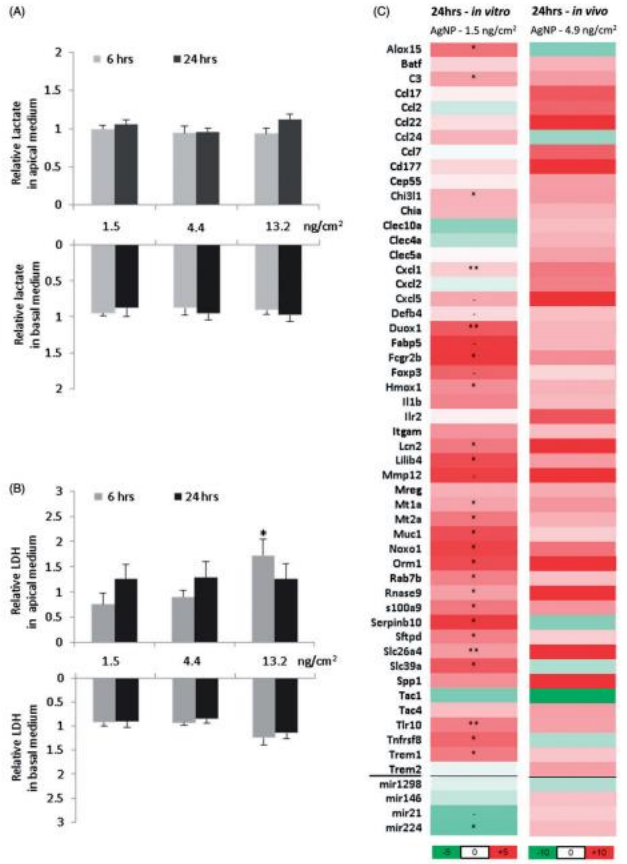


Figure 5. Exposure of small airway epithelium to AgNP aerosol. Human primary small airway epithelial cell cultures (SmallAIR) were exposed to AgNP aerosols using an AE-ALI deposition device. After 7, 20 or 60 min of exposure (respective doses 1.5, 4.4 and 13.2 ng/cm^2), cultures were incubated for 6 or 24 h and apical wash and basolateral media was assessed for levels of lactate (A) or LDH (B). RNA was also assessed for relative expression of 50 mRNA and 4 miRNA transcripts displayed as fold over control values for 7 min exposure (C; left panel). For comparison, *in vivo* lung AgNP transcript fold change alterations from RNA-SEQ data were also included (C; right panel). All AgNP measurements were normalized to air-exposed control cultures. Statistical significance was calculated between unexposed and exposed conditions using paired *t*-test ($^{\sim}p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.01$).

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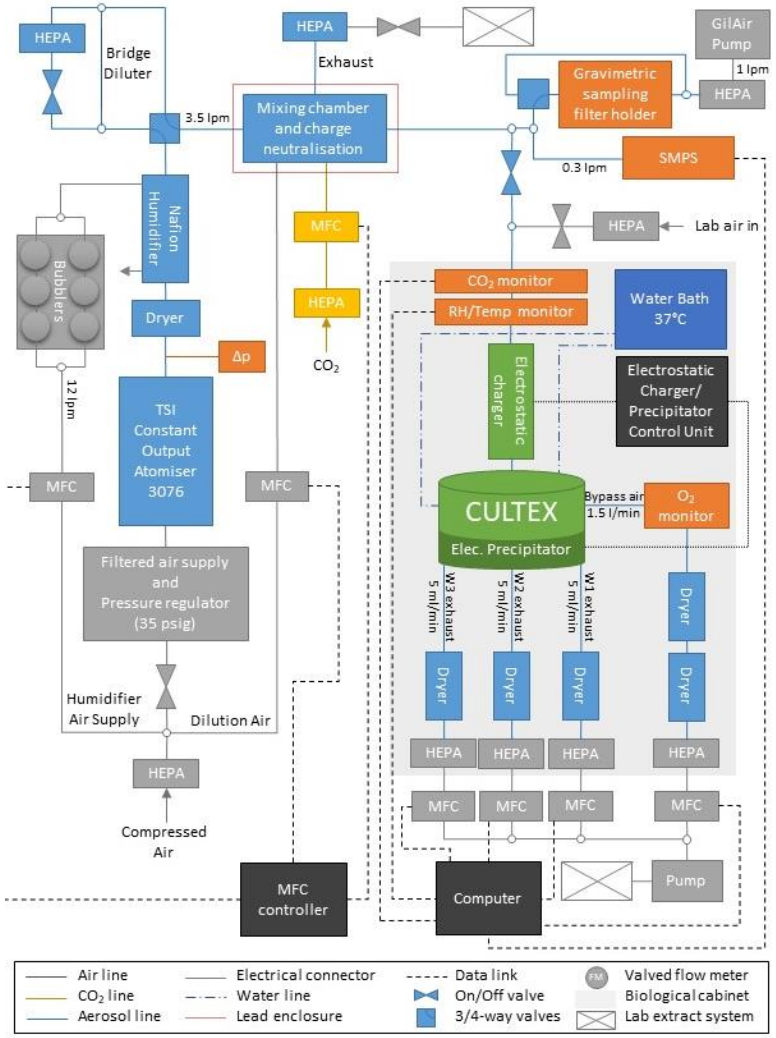
Aerosol Exposure Air-Liquid-Interface (AE-ALI) system



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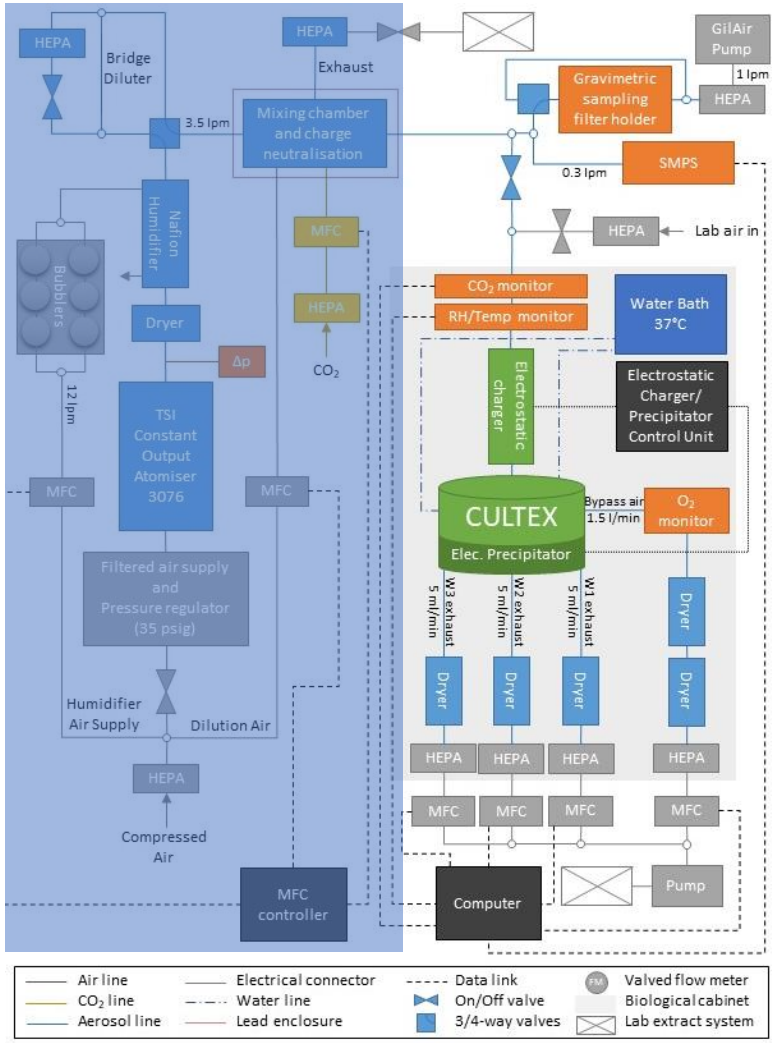
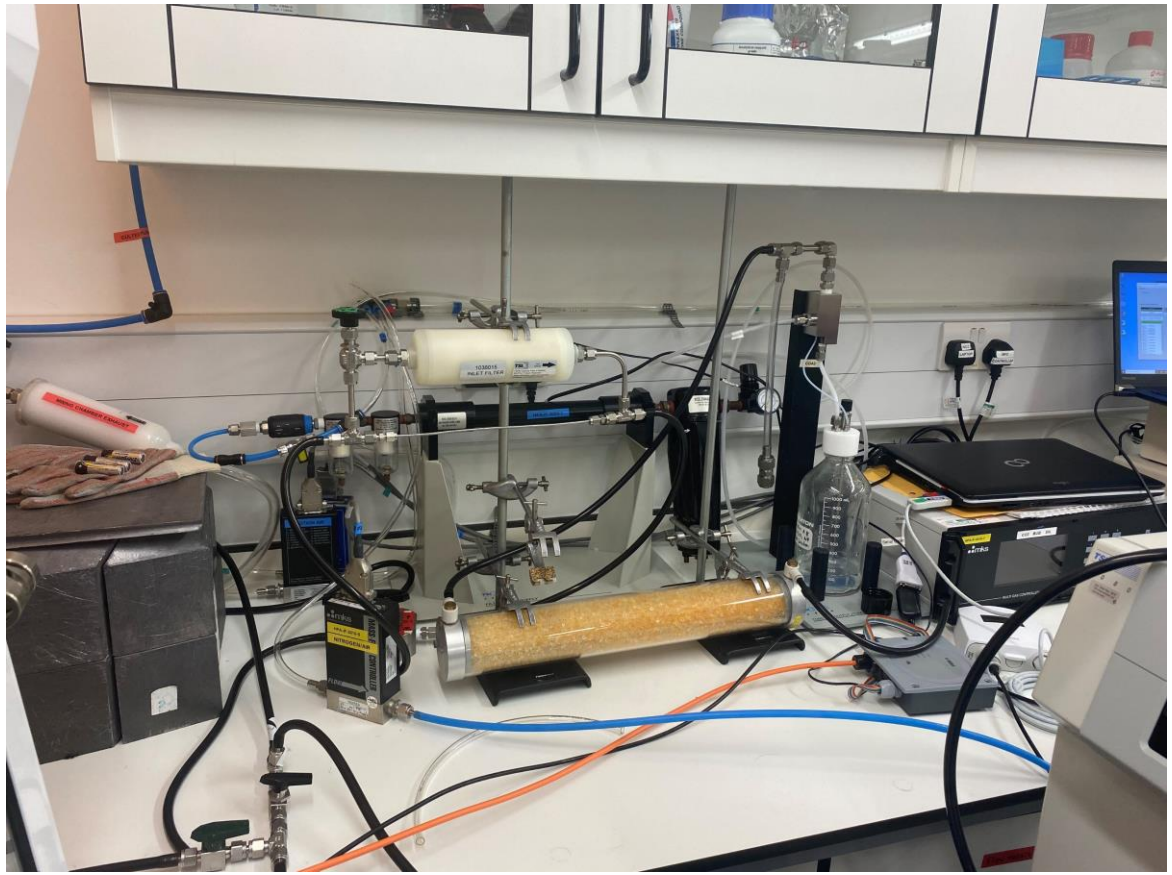


Aerosol generation and conditioning



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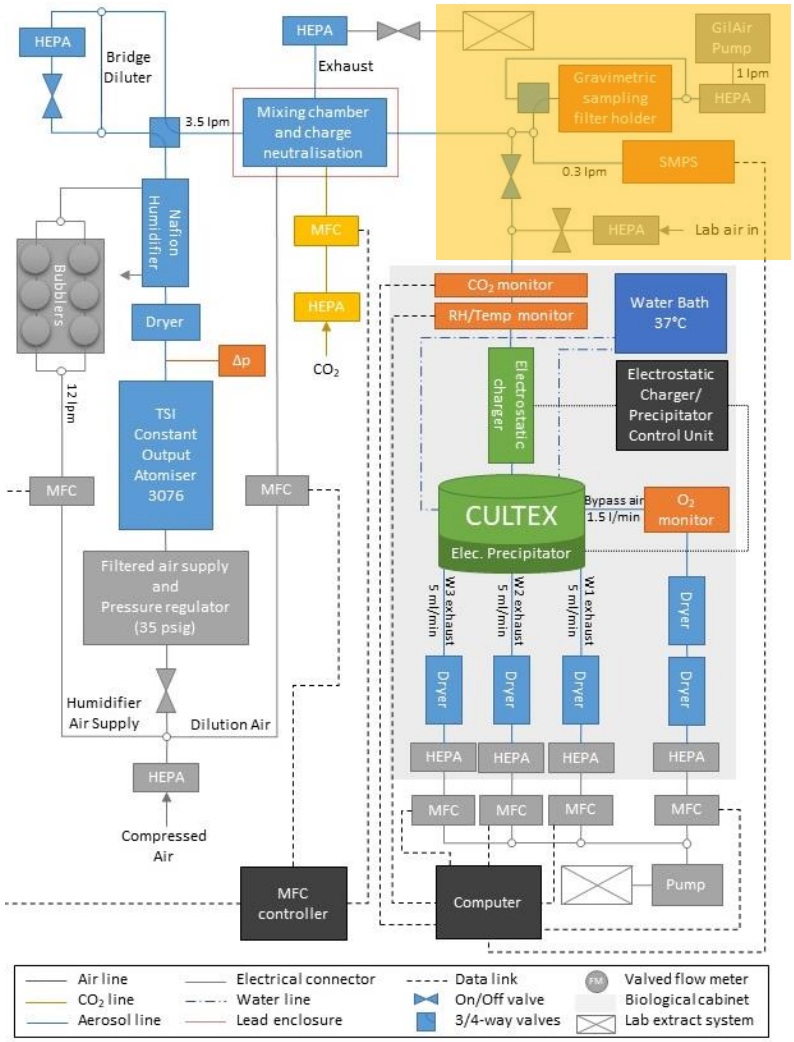
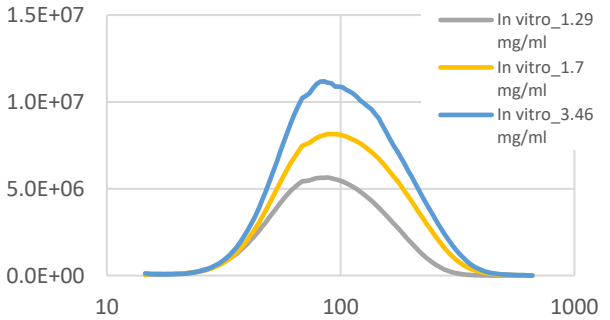
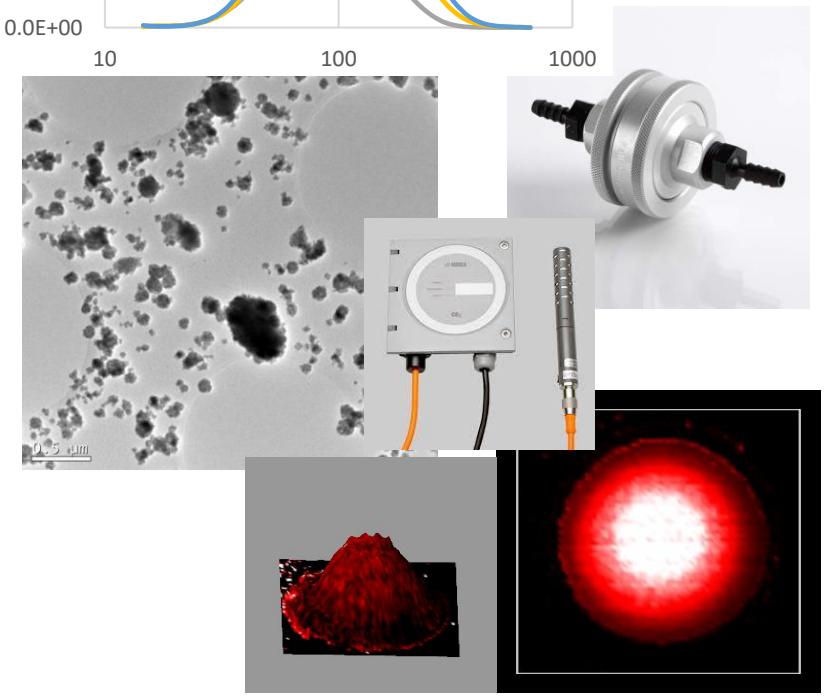


Aerosol Characterisation and Gas Concentration Measurements

- Number-based aerosol size distribution – SMPS
- Average aerosol mass concentration – Gravimetric Sampling
- Deposited mass – ICP-MS
- Images of aerosol particles and deposited particles – TEM sampling
- Deposited particle distribution – Laser Ablation ICP-MS
- O2 and CO2 concentration, gas temperature and relative humidity, system pressure





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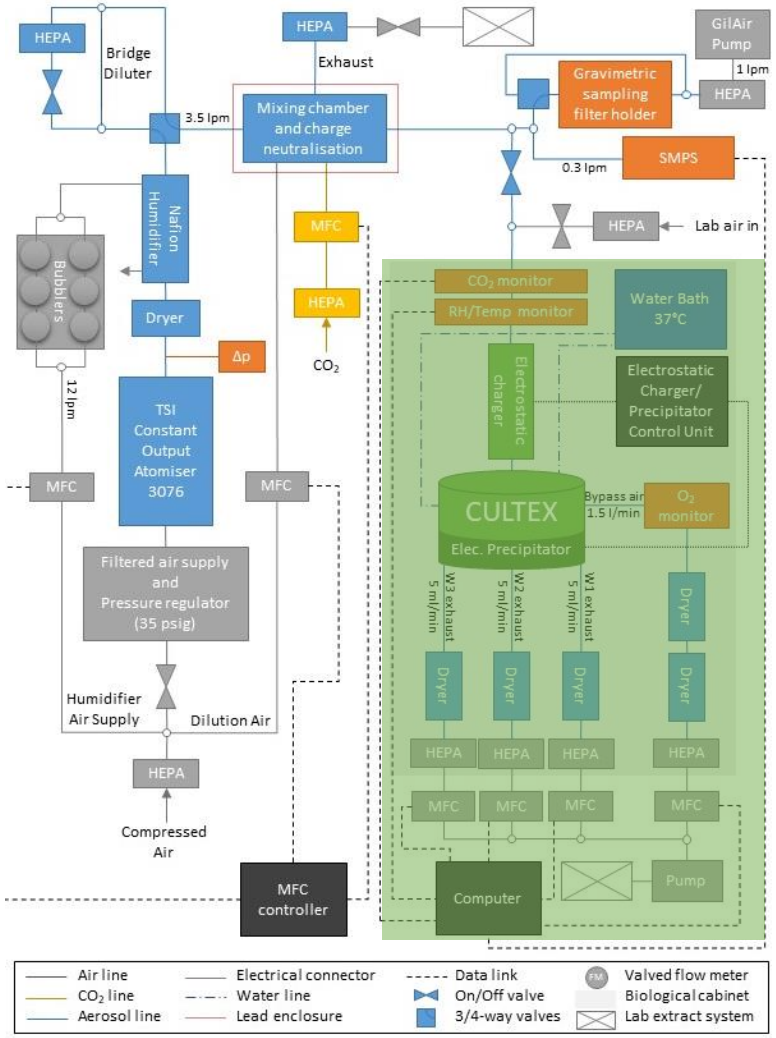
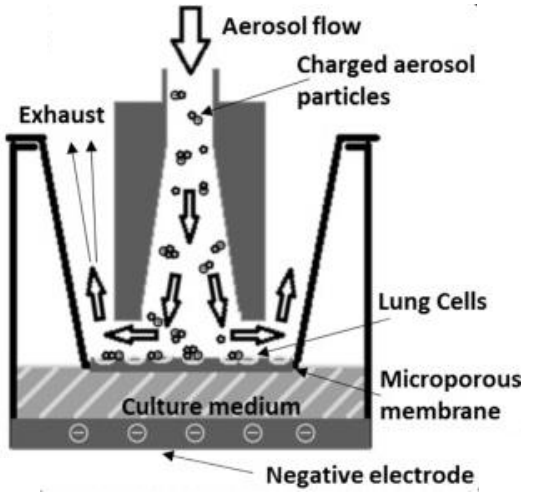


Cell Exposure - CULTEX©

- 3 wells
- 3 sizes possible – 6.5 mm, 12 mm and 24 mm Transwell inserts
- Heated well
- Guided aerosol
- Electrostatic precipitation to enhance deposition



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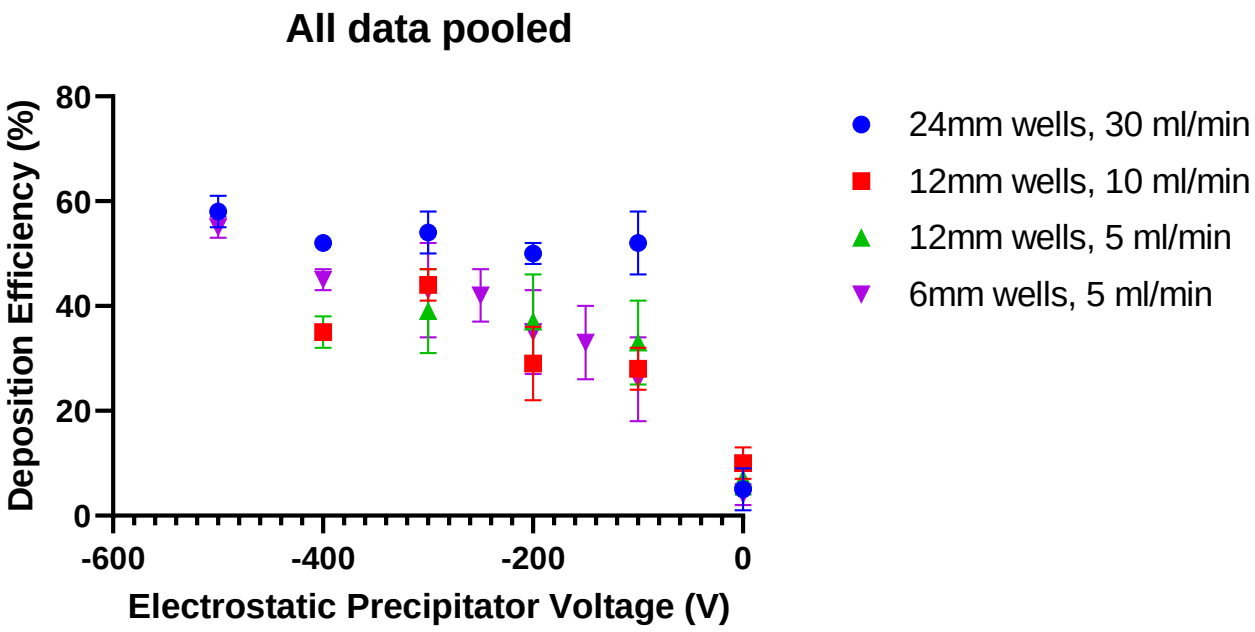
Estimation on deposition efficiency

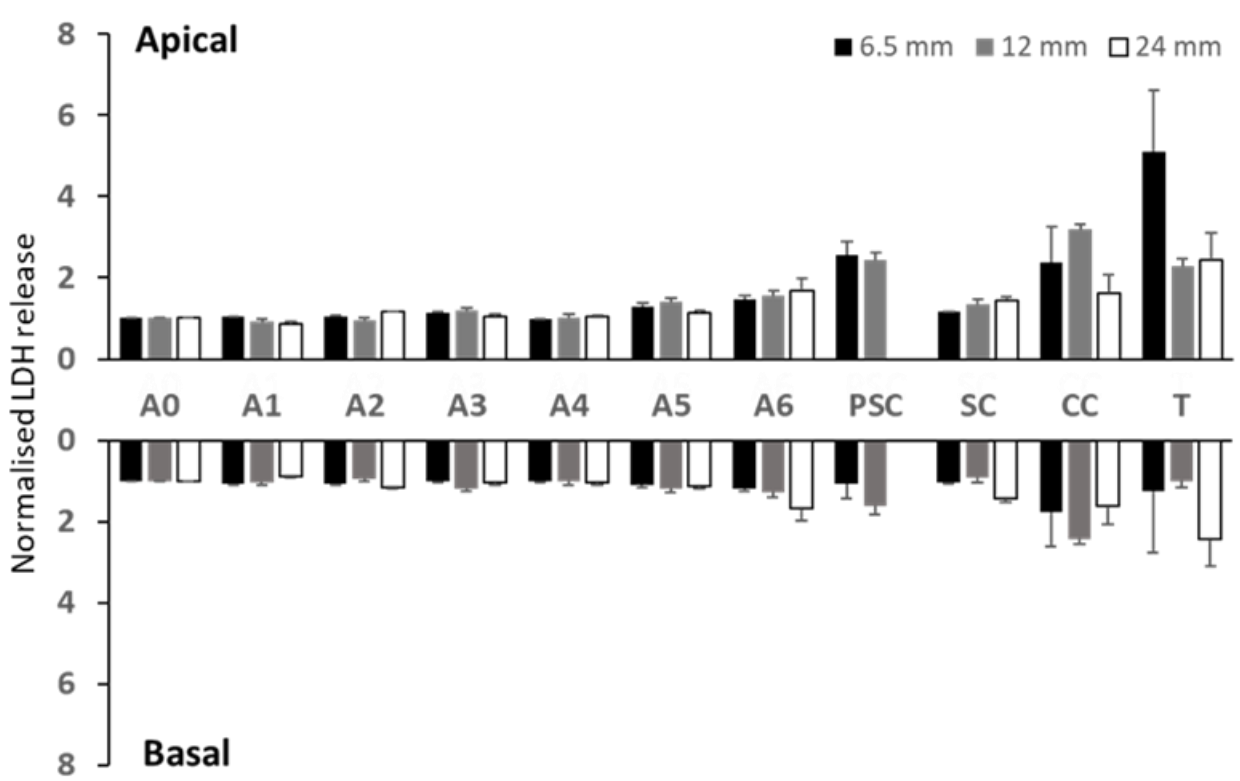


- Deposition efficiency is calculated from the aerosol mass concentration, exposure time and aerosol flowrate, and deposited mass.
- Increase in deposition efficiency with magnitude of electrostatic precipitator voltage
- For the 4 well size/flow conditions investigated not much difference in deposition efficiency at a given voltage other than for potentially the largest wells (24mm/6 well).
- Choice of conditions can’t just be based on the amount of material deposited though – appropriateness of well size and deposition pattern must be considered.

Cytotoxicity assessment

Exposure condition	Aerosol exposure Duration (mins)	Well flow rate (mL/min)		
		6.5-mm	12-mm	24-mm
Incubator control (A0)	0	-	-	-
Air control_1 (A1)	5	5	10	30
Air control_2 (A2)	7	5	10	30
Air control_3 (A3)	10	5	10	30
Air control_4 (A4)	5 + 5 (30 min interval)	5	10	30
Air control_5 (A5)	20	5	10	30
Air control_6 (A6)	30	5	10	30
Positive system control (PSC)	30	30	30	-
System control (SC)	30	5 then 0.05	5 then 0.05	5 then 0.05
Chemical control (CC)	-	-	-	-
Total cell lysis (T)	-	-	-	-

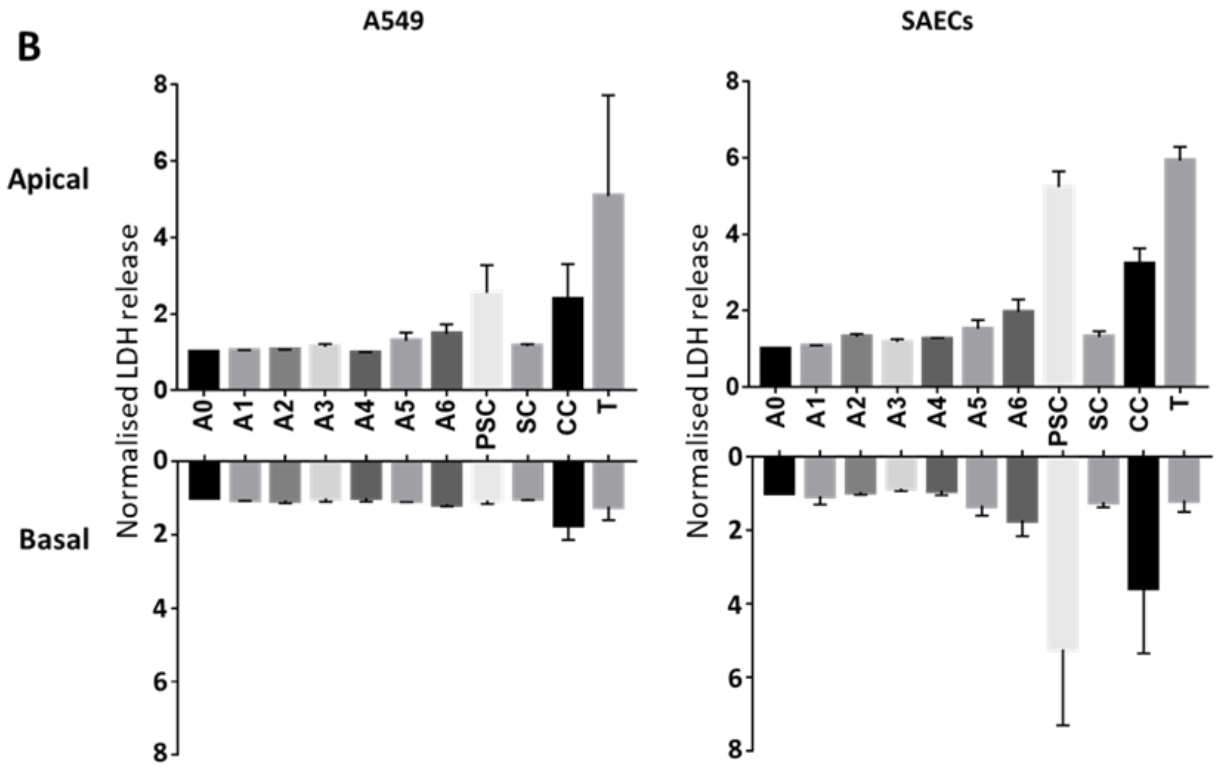
A



n=3 independent experiments on A549 cells

Cytotoxicity assessment

Exposure condition	Aerosol exposure Duration (mins)	Well flow rate (mL/min)		
		6.5-mm	12-mm	24-mm
Incubator control (A0)	0	-	-	-
Air control_1 (A1)	5	5	10	30
Air control_2 (A2)	7	5	10	30
Air control_3 (A3)	10	5	10	30
Air control_4 (A4)	5 + 5 (30 min interval)	5	10	30
Air control_5 (A5)	20	5	10	30
Air control_6 (A6)	30	5	10	30
Positive system control (PSC)	30	30	30	-
System control (SC)	30	5 then 0.05	5 then 0.05	5 then 0.05
Chemical control (CC)	-	-	-	-
Total cell lysis (T)	-	-	-	-

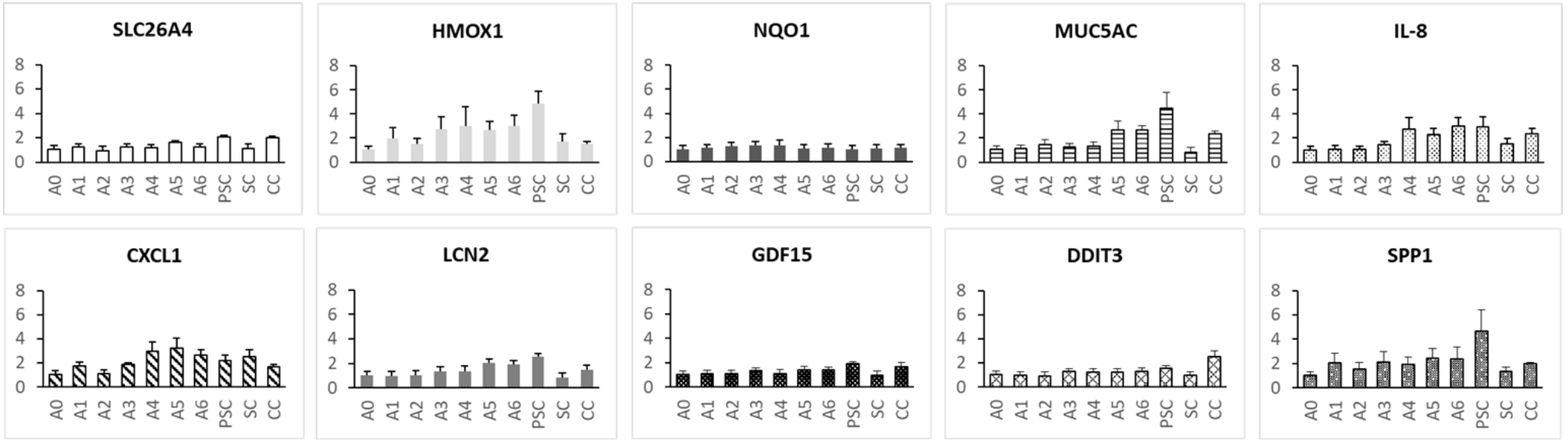


n=3 independent experiments

Expression assessment on selected genes

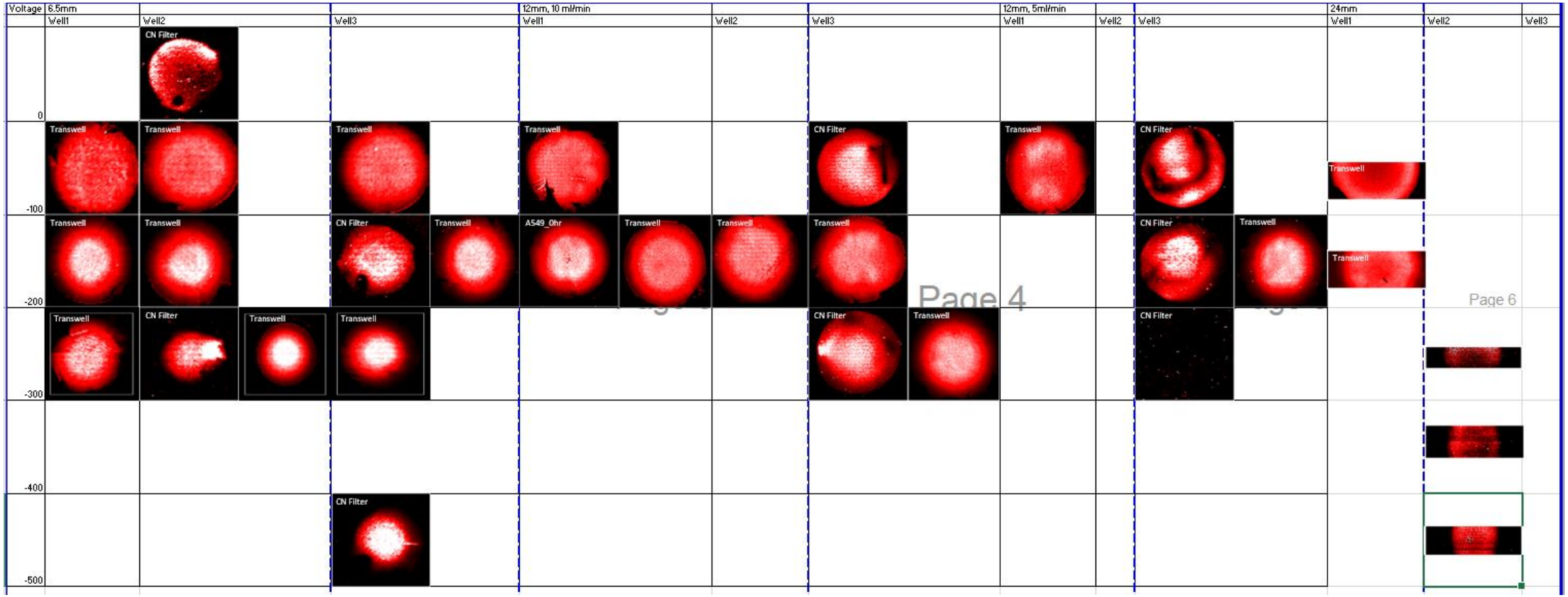
SAECs specific genes: MUC5AC and SLC26a4
Oxidative stress related genes: HMOX1, LCN2, NQO1
Inflammatory responsive genes: IL-8, CXCL1, SPP1
DNA damage related genes: DDIT3, GDF15

Exposure condition	Duration (mins)
Incubator control (A0)	0
Air control_1 (A1)	5
Air control_2 (A2)	7
Air control_3 (A3)	10
Air control_4 (A4)	5 + 5 (30 min interval)
Air control_5 (A5)	20
Air control_6 (A6)	30
Positive system control (PSC)	30
System control (SC)	30



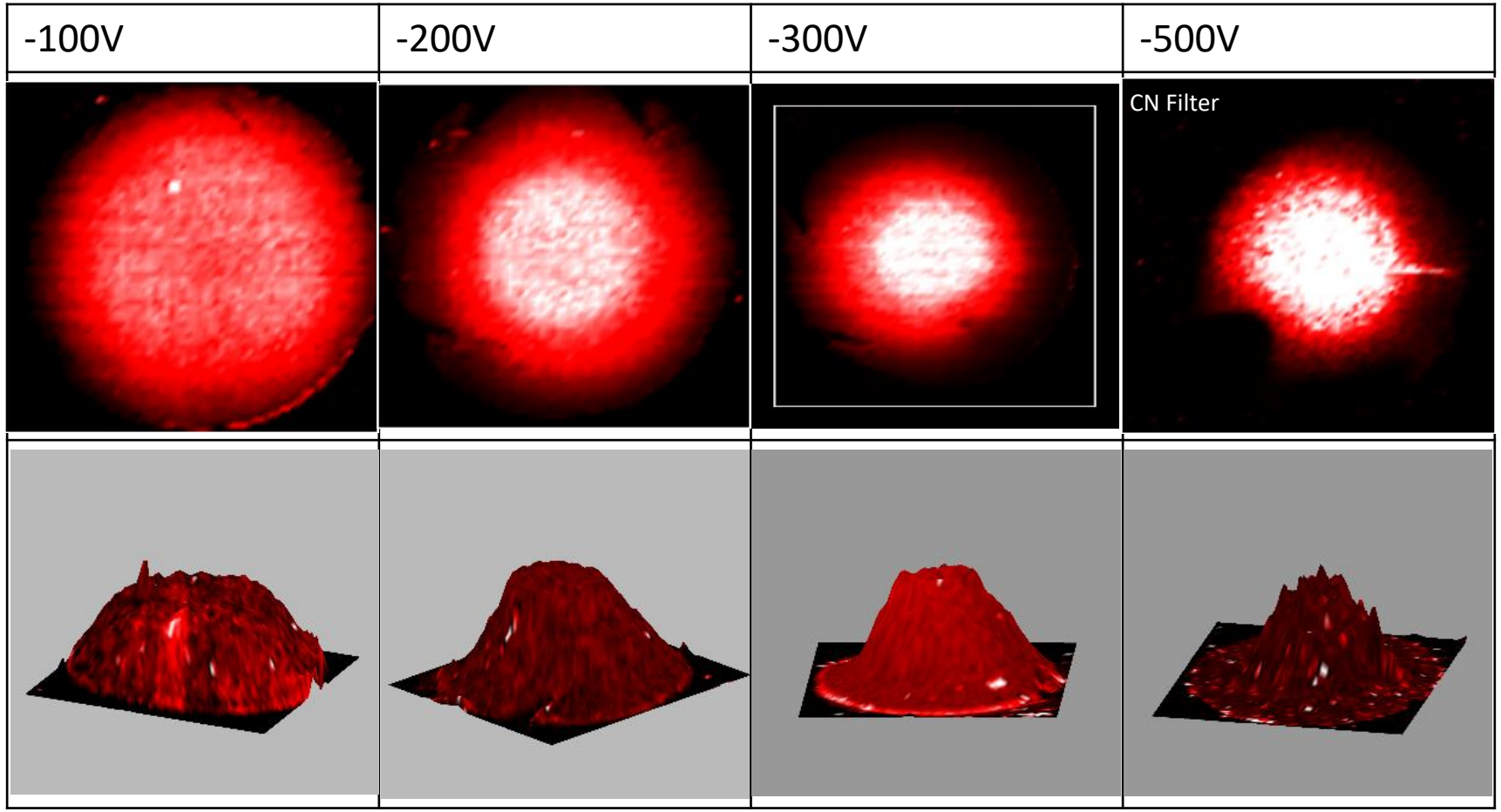
n=3 independent experiments on SAECs

Deposition pattern by ICP-MS laser ablation



Deposition pattern by ICP-MS laser ablation

6.5mm Transwell inserts, 5 ml/min



Taken together

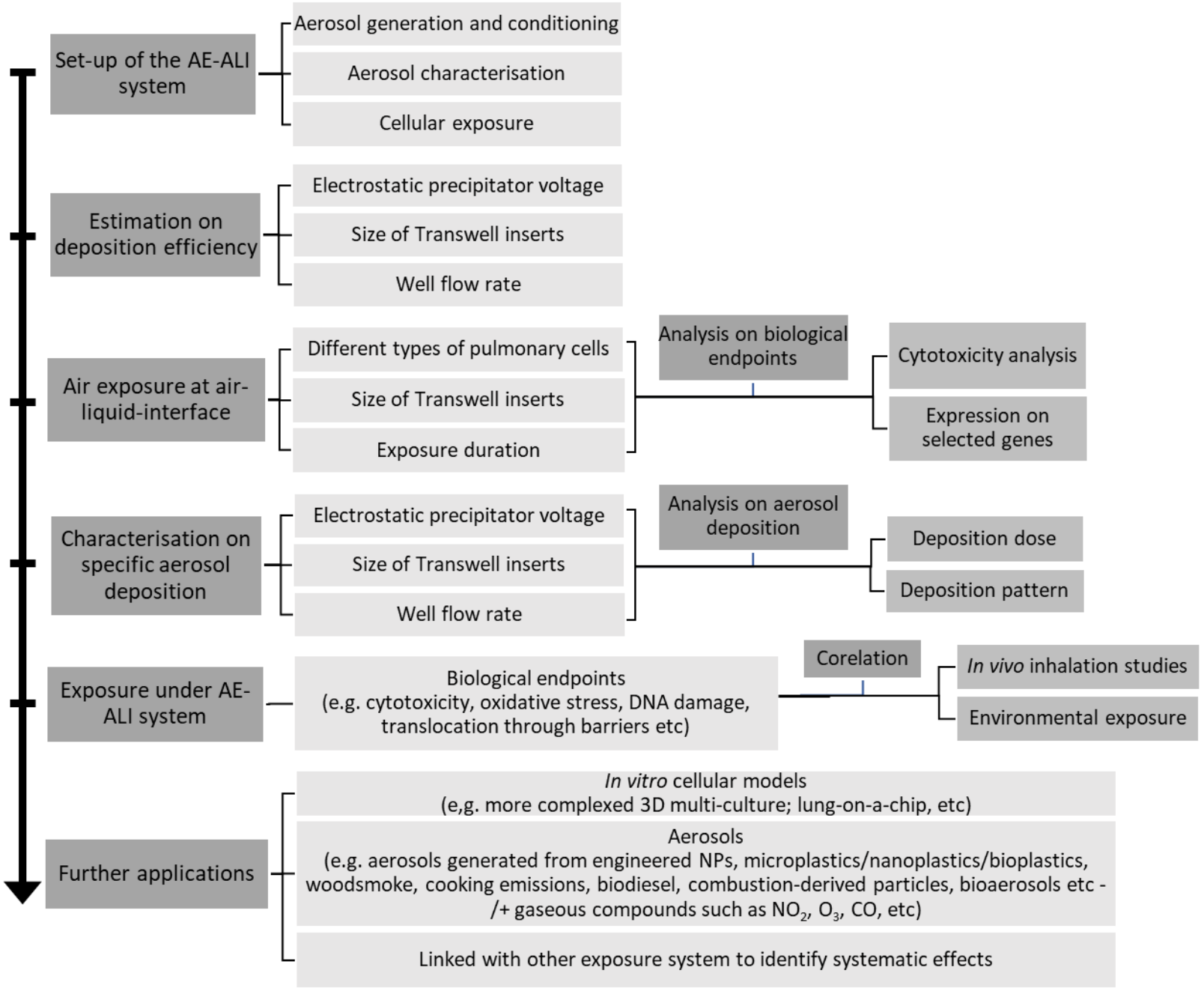


Figure. Diagram of validation of AE-ALI system

Conclusions

- Exposure duration had a significant impact on cell cultures.
- Appropriate choice of operating parameters could produce broadly uniform aerosol deposition.
- Detailed characterisation of AE-ALI systems is essential prior to use.
- Limitations (exposure dose, sensitivity of cellular models, complexity to use, etc).
- Applications and further development of this AE-ALI system
  - Test substances
  - Biological test systems
  - Linked with other exposure system?
- The results here and further applications would improve the standardisation of *in vitro* inhalation toxicity measurements.

Thanks for your listening!