Direct, Air-to-Cell Deposition of Coarse Ambient Particulate Matter Increases the Inflammatory Response In Vitro

John Volckens[1], Glenn Walters[2], Lisa Dailey[3], and Robert Devlin[3]

(1) Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins CO USA;

(2) University of North Carolina, Chapel Hill NC USA; (3) U.S. EPA, Chapel Hill NC USA

Colorado State University

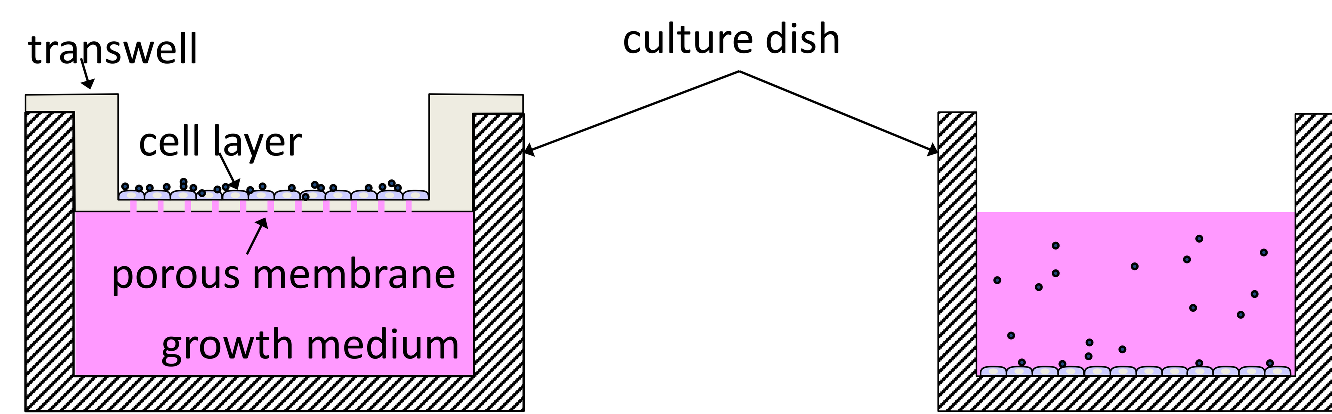
Knowledge to Go Places

I. Abstract

The objective of this work was to develop a more physiologically relevant model of particulate matter (PM) deposition to the human lung *in vitro.* Our central hypothesis is that the toxicology of inhaled particulate matter depends strongly on both the particulate dispersion state and the mode of delivery to cells. Our model employs a combination of unipolar charging and electrostatic force to deposit particles onto cells grown at an air-liquid interface in a heated, humidified exposure chamber. We show that cells exposed in this manner to concentrated, coarse ambient particulate matter express increased levels of inflammatory markers (mRNA) at 1 hour following exposure. More importantly, these effects are seen at levels (characterized by deposited mass per tissue area) that are an order of magnitude lower than effects seen with traditional in vitro methods (i.e., delivery of filter-extracted PM to cells grown submersed in medium). *Disclosure: The authors have no conflicts or relationships to declare.*

II. Materials and Methods

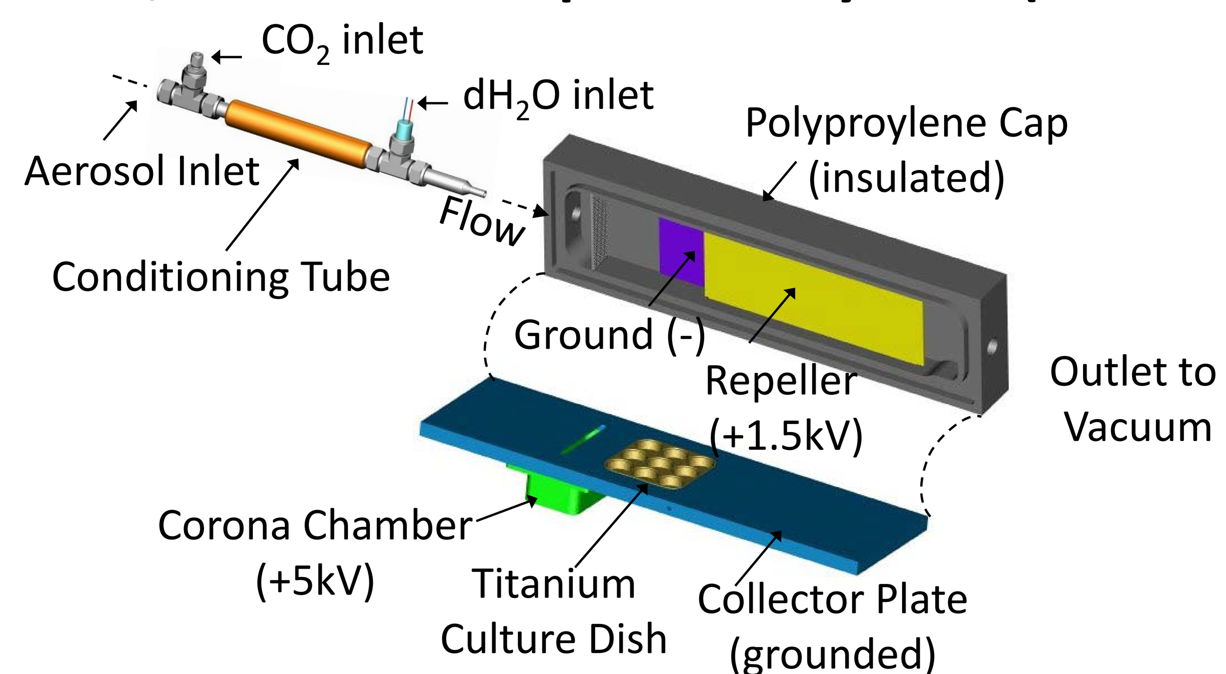
Evaluation of Two Cell Culture/Exposure Systems:



(A) Direct-Air Deposition: Particles deposited directly onto cells grown at an air-liquid interface

(B) Indirect-Liquid Deposition: Particles collected on filters, extracted, resuspended in medium and applied to cells submersed in a plastic Petri dish

Direct, Air-to-Cell Deposition System (EAVES2)



The electrostatic aerosol in vitro exposure system (EAVES2) is a second-generation design.[1] Aerosol entering the chamber is positively charged by a corona and then electrically precipitated onto Transwell inserts (9 total).

Exposure to Ambient PM_{coarse}

Chapel Hill, NC

Ambient, coarse PM (defined as having aerodynamic diameters between 2.5 and 10 μm)

Virtual impactor concentrates coarse aerosol fraction by 20-30x with filter samples collected in parallel

Nine, 3-hr exposures:
3 phenotypes
3 exposures/donor
3 replicates per test

EAVES2 Device

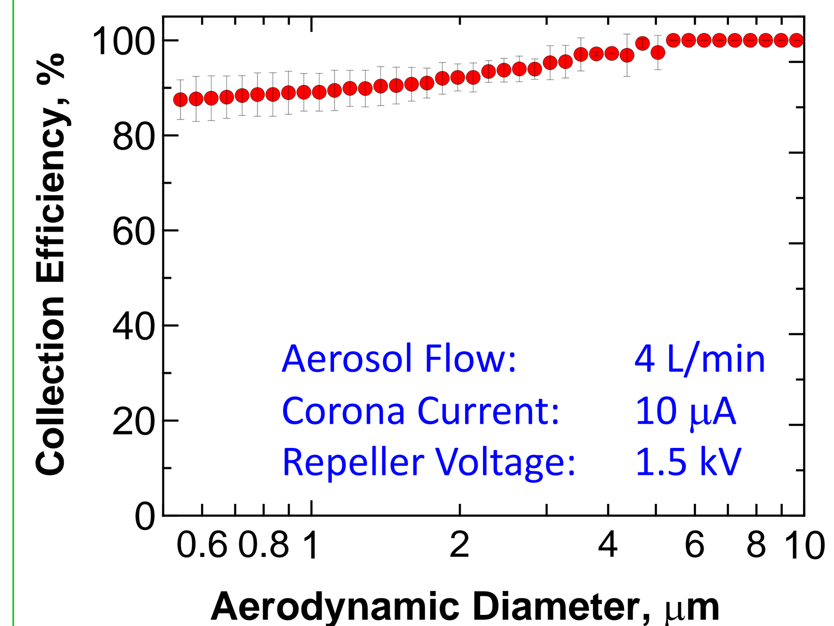
Genes of Interest:
Heme Oxygenase 1 (HOX-1)
Cyclooxygenase 2 (COX-2)
Interlukin 8 (IL-8)
Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)

Cell Culture and Analysis

Normal human bronchial epithelial cells obtained by brush biopsy from healthy volunteers. Passaged twice in plastic and then plated on collagen-coated Transwell membranes (Snapwell, Corning Inc.). Cultures were taken to air-liquid interface and maintained for a minimum of 3 days prior to exposure.[2] Total RNA isolated from cells (RNeasy, Quiagen) and stored at -80ºC. RNA was quantified (NanoDrop, Agilent) and converted to cDNA using random hexamers. Transcripts identified using real-time RT-PCR (Taqman, Applied Biosystems) and normalized to GAPDH concentrations. LDH assay performed using Cytotox 96 (Promega).

III. Results

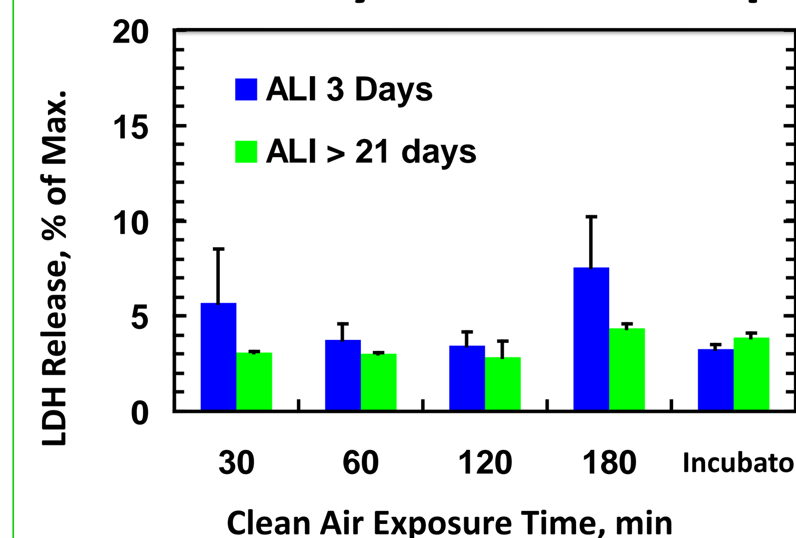
Particle collection efficiency



Coarse-mode aerosol is efficiently collected by the EAVES2 unit.

Each cell culture well receives approximately 1.5% of the total aerosol mass entering the device.

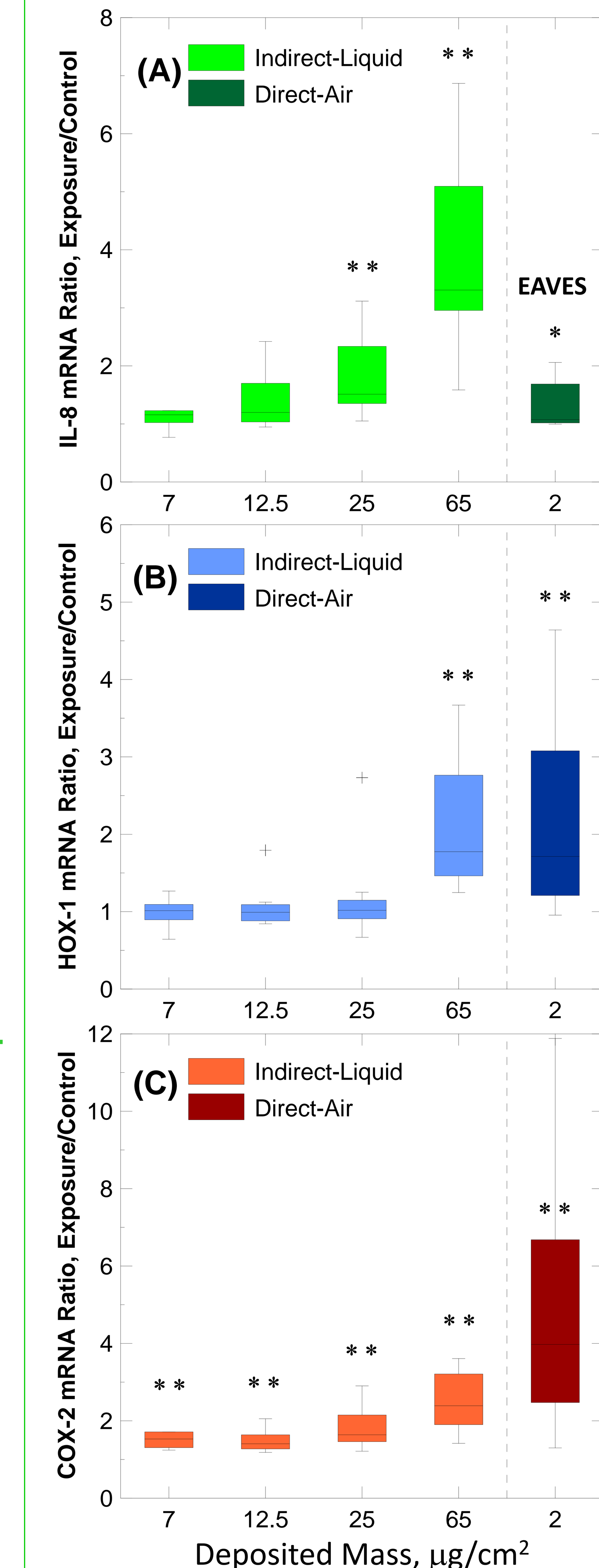
Cell Viability: Clean Air Exposure



Apical release of lactate dehydrogenase (**LDH**) is a measure of membrane integrity and cell viability.

Cells appear viable for up to 3 hours of clean air exposure in the EAVES2.

Transcriptional markers of inflammation



Box-whisker plots of mRNA expression profiles (ratio of exposure to control) at 1-hour post exposure. Light-shaded boxes represent cells grown submersed and exposed to liquid extracts of PM collected on filters. Dark boxes represent cells grown at an air-interface and exposed directly to coarse PM using the modified EAVES system. The (+) symbols indicate data outliers, the (*) indicates significance at $p < 0.05$ and (**) at $p < 0.01$.

For HOX-1 and COX-2, An exposure of 2 μg of particulate mass deposited via direct-air deposition using the EAVES technology produces the same level of mRNA accumulation as 65 μg of mass deposited using the indirect system with a syringe spike.

IV. Discussion

Traditional methods for examining the toxicology of particulate matter *in vitro* have used extracts of PM collected on filters and then applied to cell cultures via syringe spike. There are several drawbacks to such a technique. First, the extraction media may cause physical and chemical changes to the PM. Second, particle agglomeration is likely, which reduces the amount of surface area presented to cells. Third, the bolus addition of particles in solution makes quantification of 'dose' difficult, as not all suspended particles will reach the cell surface during the exposure. Finally, the exposure does not mimic the deposition *in vivo*, which occurs an air-liquid interface. The inflammatory responses seen above are only evident with 'syringe-spike' methods at an order of magnitude higher levels.

V. Model Limitations

The use of corona charging and electrical precipitation has several drawbacks. Ozone is generated by the corona (80 ppb) may sensitize cells – although both control and exposure cells are affected. Second, charged particles *may* be more toxic than neutral ones, although preliminary investigation has not indicated so.[1] Third, the presence of an electric field above the cells may induce stress, although that, too, has yet to be shown relative to incubator controls. Further work will investigate and potentially mitigate these issues.

References: (1) DeBruinje et al. Inhalation Toxicology. 21, 91-101 (2009).
(2) Ross, et al. Am. J. Respiratory Cell and Molecular Biology. 37, 169-185, (2008).
Acknowledgement: This research was supported by grant #5K25ES014378 from the NIEHS

Contact: Dr. John Volckens
1681 Campus Delivery, Colorado State Univ.
Fort Collins CO, 80521 john.volckens@colostate.edu