Pro-inflammatory responses to PM0.25 from airport and urban traffic emissions at submerge cell culture condition: A comparison with air-liquid interface (ALI) culture

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Contents

• The cytotoxicity of aviation and urban traffic PMs under submerge exposure

• Air-liquid interface (ALI) exposure and exposure systems
Humans are constantly exposed to various substances ranging from industrial gases, traffic emissions to cigarette smoke.

The adverse effects: Chronic respiratory disease and lung disease; Neurotoxicity
Study the inhalation exposure

Two ways: Epidemiology and Toxicology

Toxicology is traditionally based on in vivo experiments

There is an increasing request to use in vitro models

Cell model: 16HBE (human bronchial epithelial) cell easy to culture and grow fast

Particles Deposition

Gas exchange

Structures of the respiratory tract (BéruBé et al 2010)
Background:

Aviation industry and airport traffic increase fast in recent years; Many large airports are located near urban area, which may have a significant impact on our environment and health.

Few information on sources to airport PM emissions and cytotoxicity of airport PMs

Los Angeles International Airport (LAX) Hudda et al Environ Sci Technol. 2014
Exposure materials and method

**Materials:**

- **PM$_{0.25}$ samples** (5 LAX, 5 USC, 1 turbine and 1 diesel samples)
- 5 airport samples from Los Angeles International Airport (LAX).
- 5 urban traffic samples from a freeway around University of Southern California (USC)
- 1 turbine and 1 diesel samples from turbine and diesel engine
Submerged exposure:

- Particles were extracted from filters, the suspension is added in culture medium, and exposed to cells for 4 hs.
- After 4 hs, medium was refreshed for 20 hs recovery.

Measurements

1. Measure elemental composition and oxidative potential of PM samples
2. In vitro tests including cell viability, ROS activity and inflammatory responses after exposure
Results – Elemental contributions

1. Airplane emission was the major contributor to airport PMs, following by road dust and traffic emissions
2. Urban traffic PMs have multiple comparable contributors including traffic emissions, suspended road dust and atmospheric secondary sulfates.

Aircraft emission = S
Ocean spray = Na
Road traffic emissions = Mn, Fe, Cu, Zn, Ba, Pb, Ni, Mg
Road/Soil dust: Al, K, Ca, Ti
## Results – oxidative potential

the redox activity induced by heavy metals in particle samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Geometric mean of oxidative potential (AA and ESR) values.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>AA(nmol AA/s/μg)</td>
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<tr>
<td>LAX (n=5)</td>
<td>0.33±0.10</td>
</tr>
<tr>
<td>USC (n=5)</td>
<td>1.14±0.18</td>
</tr>
<tr>
<td>Negative control (dH₂O)</td>
<td>0.08±0.02</td>
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<tr>
<td>Positive control (DOFA)</td>
<td>1.36±0.13</td>
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</tbody>
</table>

Compared to USC samples, LAX samples seem less reactive

**Lower oxidative potential (lower heavy metals)**
Results- intracellular ROS generation

Average ROS activity after 1, 2, and 4 h exposure with 10 μg/mL PMs and 20 h recovery

LAX samples induced lower ROS generation compared to USC
Results - inflammatory responses

**LAX samples induced higher level of inflammation during 20 h recovery**

Translocated particles in cells play an important role in inflammatory responses during 20 h recovery.

Size distribution:
- Mean Particles diameter (LAX) $\approx 20$ nm
- Mean Particles diameter (USC) $\approx 35$ nm

IL-6, IL-8, and TNF-α expression after 20 h recovery

Translocation of particle into cells
(Andre Nel et al; Nature Material. 2009)
Conclusions

- Airplane emission was the major contributor to airport PMs; Urban traffic PMs have multiple contributors.
- LAX samples seem less reactive compared to downtown USC
  - Lower oxidative potential and ROS generation
- LAX samples were more potent in inducing inflammation

Airport PMs showed similar toxic properties to the urban traffic PMs
Improvements

Conventional Submerged exposure

Submerged exposure
Deposition mass?

Shortcomings:
1. Deposited particles remains unknown
2. Characteristics of particles can be altered
3. Can not be used for gases and aerosol real-time exposure
**Air-Liquid Interface culture**

**ALI culture:** Cells are cultured on the *apical membrane* of insert *without covered medium*. Culture medium is added to the *basolateral side*.

The setup simulates the human airway conditions and stimulates the cell differentiation (mucus release and cilia formation)
Air-Liquid Interface Exposure

ALI Exposure

1. Real-time exposure
2. No loss
3. Realistic
ALI exposure systems

**VITROCELL® automated exposure station**

Various materials transferred into exposure cabinet and passed over cells by **continuous air flow**
ALI exposure systems

VITROCELL® Cloud exposure system

Aerosol generator
Exposure Cabinet
Temperature control

Microbalance

Control  Exposure

Phase 1  Emission Of Cloud
Phase 2  Homogeneous Mixing
Phase 3  Gravitational Settling

Droplet sedimentation mechanism
## Comparison – exposure systems

<table>
<thead>
<tr>
<th><strong>Advantages</strong></th>
<th><strong>Disadvantages</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Different materials exposures</td>
<td>1. Airflow might be harmful to cells</td>
</tr>
<tr>
<td>2. More realistic (continuous exposure)</td>
<td>2. Less cells can be selected</td>
</tr>
</tbody>
</table>

### VITROCELL® automated exposure station

**Advantages**

1. User-friendly
2. More cells can be selected
3. Less volume of suspension

### VITROCELL® Cloud exposure system

**Advantages**

1. Different materials exposures
2. More realistic (continuous exposure)

**Disadvantages**

1. Only for suspension exposure
2. Less realistic (one-time exposure)
3. Large volume of suspension is required
Selection of cell models under ALI conditions

Cells: **16HBE, Calu-3, H292 and BEAS-2B cells**
Tests: the integrity of cell membrane (TEER, ZO-1 protein staining, and LDH leakage)
Toxicity of UFPs from Amsterdam airport and urban traffic emissions under ALI exposure
Acknowledgement

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