Comparing Nanomaterial Toxicity with Lung Cells Cultured under Air-Liquid Interface and Submerged Conditions

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Cell exposure at the Air-Liquid Interface

Submerged exposure

Air-liquid interface exposure

Physiological realistic testing!

Lenz et al., Am J Respir Cell Mol Biol., 2014
Cell exposure at the Air-Liquid Interface

4-5 µm

Rajiv Dhand 2004


https://www.youtube.com/watch?v=1KdkmgnmzWY
https://www.youtube.com/watch?v=1lA_SSScMeE
Suspension preparation protocol

Nanopowders → Suspended in pure water → Vortex shaker

(NaCl)

Dynamic light scattering → Sonication

CPC Comprehensive Pneumology Center

HelmholtzZentrum münchen German Research Center for Environmental Health
Uniform & fast delivery of NM aerosols

Fluorescein Concentration

Nebulization

Fluorescein Concentration (plate reader)

QCM deposition

Mean deposition: 49.5%
Insert-insert variability: 3.4%
## Cell growth and exposure conditions

<table>
<thead>
<tr>
<th></th>
<th>Submerged</th>
<th>Sub-Insert</th>
<th>ALI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plate type</strong></td>
<td>6-well plate</td>
<td>6-well insert</td>
<td>6-well insert</td>
</tr>
<tr>
<td><strong>Cell type</strong></td>
<td>A549 IL-8</td>
<td>A549 IL-8</td>
<td>A549 IL-8</td>
</tr>
<tr>
<td>Seeding surface</td>
<td>9.6 cm²</td>
<td>4.2 cm²</td>
<td>4.2 cm²</td>
</tr>
<tr>
<td>Seeding Nr.</td>
<td>1 mil.</td>
<td>1 mil.</td>
<td>1 mil.</td>
</tr>
<tr>
<td>Growth time</td>
<td>3 day</td>
<td>4 day</td>
<td>4d+1d (in air)</td>
</tr>
<tr>
<td>Apical Med.vol.exp.</td>
<td>1 ml</td>
<td>1 ml</td>
<td>0 ml</td>
</tr>
<tr>
<td>Med. Height</td>
<td>ca. 1 mm</td>
<td>ca. 2.4 mm</td>
<td>0.01 mm (ZnO suspension)</td>
</tr>
<tr>
<td><strong>Nanomaterial</strong></td>
<td>ZnO NM110</td>
<td>Type</td>
<td>Size, TEM</td>
</tr>
<tr>
<td></td>
<td>50-150 nm</td>
<td>12 m²/g</td>
<td></td>
</tr>
</tbody>
</table>
Cell viability (WST-1 assay), 24h

Viability, % of control vs. Dose, cm²/cm²

- **Air-liquid Interface**
- **Submerged-Conventional**
- **Submerged-Insert**

Dose, cm²/cm²

0 0.5 1 1.5 2 2.5

Viability, % of control

0% 20% 40% 60% 80% 100% 120% 140%

(20 µg/cm²)
(80 µg/ml)
Cytotoxicity (Lactate Dehydrogenase release), 24h

Cytotoxicity, % of high control

Dose, cm²/cm²

Air-liquid Interface

Submerged-Insert

Submerged-Conventional

(20 µg/cm²)

(80 µg/ml)
Interleukin-8 induction, 24h

- **Sub-Insert**
- **Air-Liquid Interface**

**Graph:**
- Y-axis: IL-8 release, fold of control
- X-axis: (Nominal) dose, cm²/cm²

- Data points for Sub-Insert:
  - 0 (Nominal) dose: 0 fold of control
  - 0.5: 2 fold of control
  - 1: 15 fold of control
  - 1.5: 30 fold of control
  - 2: 25 fold of control
  - 2.5: 0 fold of control

- Data points for Air-Liquid Interface:
  - 0 (Nominal) dose: 0 fold of control
  - 0.5: 2 fold of control
  - 1: 10 fold of control
  - 1.5: 20 fold of control
  - 2: 5 fold of control
  - 2.5: 0 fold of control

**Notes:**
- Sub-Insert data points show a significant increase in IL-8 release with increasing dose.
- Air-Liquid Interface data points show a less pronounced increase in IL-8 release.

**Additional Information:**
- Nominal dose: (20 µg/cm²)
- Concentration: (80 µg/ml)

**Institution:**
- CPC Comprehensive Pneumology Center
- HelmholtzZentrum münchen German Research Center for Environmental Health
Calculation of Sedimentation

(Bulk density: 5.6 g/cm³)

Effective density

- 4.0
- 3.5
- 3.0
- 2.5
- 2.0
- 1.5 g/cm³

Sedimentation distance, mm

ZnO agglomerate size, nm

TEM

DLS

ca. 250 nm

*Colloid and Surface Chemistry, Duncan J. Shaw, 4th edition, 1992 p.21-25
Summary

I. Uniform and efficient aerosol delivery was achieved using VITROCELL- CLOUD (ALICE-CLOUD) with 5 min exposure time. Suspension quality is controlled for aerosolization.

II. Cell viability decreased and LDH/IL-8 induction increased when dose increased.

III. ZnO nanoparticle dose-response curve of lung epithelial cells varied for submerged (sharp) and air-liquid interface (broad) exposures.

IV. Cells grown in plastic surface (well) and at porous insert surface behaved differently:

   I. dose rate

   II. Cells are more sensitive in plastic wells

   III. both
Thank you!

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Clara Wimmer
Paula Mayer

VITROCELL Systems GmbH, Dr. Tobias Krebs
Reference

Vitro-cell ALICE video: https://www.youtube.com/watch?v=1KdkmqmcxWY


Aeroneb video: https://www.youtube.com/watch?v=1lA_SSScMeE