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Overview

- Introduction
- Materials & methods
- Results
- Conclusion
Introduction

- Air pollution
  - Epidemiological studies:
    association between particulate air pollution and respiratory & cardiovascular mortality / morbidity
  - most important:
    ultrafine particles = PM$_{0.1}$ $\phi < 0.1$ μm
- Nanotechnology: nanoparticles (<0.1 μm)
Introduction

- In vitro: ?

**In vitro pulmonary translocation model**

- screen particles for translocation
- what is the mechanism
Cell culture model

Transwell clear insert

Cell monolayer

Apical compartment

Basolateral compartment

Pores: 0.4 µm or 3 µm
Materials & Methods

- **Cell types**
  - A549: human alveolar epithelial cell line
  - Calu-3: human bronchial epithelial cell line
  - Primary rat Type II pneumocytes
Materials & Methods

- Control integrity of monolayer
  - TEER: Trans-Epithelial Electrical Resistance ($\Omega\cdot\text{cm}^2$)
Materials & Methods

- Sodium fluorescein leakage (1mg/ml, 1h)
  leakage <1% = tight monolayer (Agu, et al. 2001)

? Relation between TEER and sodium fluorescein leakage
Materials & Methods

- Translocation studies
  - Particles: fluorescent polystyrene latex-beads
    $\varnothing = 0.046 \, \mu m$
    Carboxyl or amine-modified
  - Apical compartment: medium without serum + 1% BSA + particles (2.5 $\mu$g)
  - Incubation time: 14-16h
  - Detection: Fluorescence Correlation Spectroscopy (FCS)
Results

- Integrity of monolayer: TEER
- A549 cell line - 0.4 μm pores

Waymouth medium

![Graph showing TEER (ohm.cm²) over days for different cell concentrations.](image)
Results: TEER

- Primary Type II pneumocytes – 0.4 μm pores
- Waymouth medium

<table>
<thead>
<tr>
<th></th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 x 10^6 cells/ cm²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No coating</td>
<td>69 ± 23 Ω.cm²</td>
<td>224 ± 51 Ω.cm²</td>
</tr>
<tr>
<td>Coating#</td>
<td>24 ± 15 Ω.cm²</td>
<td>89 ± 49 Ω.cm²</td>
</tr>
</tbody>
</table>

| 0.6 x 10^6 cells/ cm² |               |               |
| No coating           | 78 ± 46 Ω.cm² | 241 ± 90 Ω.cm²|
| Coating#             | 40 ± 21 Ω.cm² | 168 ± 56 Ω.cm²|

# mono-fibrillar collagen coating
### Results: TEER

- **Primary Type II pneumocytes - 3 μm pores**
  - Waymouth medium

<table>
<thead>
<tr>
<th></th>
<th>Day 3</th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>0.6 x 10⁶ cells/ cm²</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No coating</td>
<td>7 ± 7 Ω.cm²</td>
<td>31 ± 13 Ω.cm²</td>
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<tr>
<td>Coating#</td>
<td>10 ± 7 Ω.cm²</td>
<td>25 ± 10 Ω.cm²</td>
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<tr>
<td><strong>0.9 x 10⁶ cells/ cm²</strong></td>
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<td></td>
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<tr>
<td>No coating</td>
<td>8 ± 3 Ω.cm²</td>
<td>24 ± 9 Ω.cm²</td>
</tr>
<tr>
<td>Coating#</td>
<td>13 ± 4 Ω.cm²</td>
<td>30 ± 6 Ω.cm²</td>
</tr>
</tbody>
</table>

# mono-fibrillar collagen coating
Results: TEER

Calu-3: MEM medium

0.4 μm pore size

3 μm pore size

DAY

TEER (ohm.cm²)

0.3 x 10⁶ cells/cm²

0.6 x 10⁶ cells/cm²
Results

- Sodium fluorescein leakage

? Relation between TEER and sodium fluorescein leakage
Relation between TEER and sodium fluorescein leakage

- Primary Type II pneumocytes
  - 0.4 μm pore size
  - \( r^2: 0.89 \)
  - X intercept: 2.40

minimal TEER: 251 Ω.cm²
Relation between TEER and sodium fluorescein leakage

- **Type II pneumocytes**
  - 0.4 μm: ~251 Ω.cm²

- **Calu-3**
  - 0.4 μm: ~250 Ω.cm²
  - 3 μm: ~420 Ω.cm²
Results: Translocation studies

- Control inserts WITHOUT cell monolayer
  - 0.4 μm pore size

Carboxyl-modified particles

<table>
<thead>
<tr>
<th></th>
<th>Carboxyl-modified particles</th>
<th>Amine-modified particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical</td>
<td><img src="image1.png" alt="Graph" /></td>
<td><img src="image2.png" alt="Graph" /></td>
</tr>
<tr>
<td>Basal</td>
<td><img src="image3.png" alt="Graph" /></td>
<td><img src="image4.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

μg particles
Results: Translocation studies

- Control inserts WITHOUT cell monolayer
  - 3 μm pore size

Carboxyl-modified particles

Amine-modified particles

- 0.4 μm: passage of very small amount
- 3 μm: passage of 50%-80% of particles
Results: Translocation studies

- In presence of cell monolayer
  - Type II pneumocytes
    - 0.4 μm: no passage
  - Calu-3 monolayer
    - 0.4 μm: no passage
    - 3 μm
Results: Translocation studies

- In presence of cell monolayer
  - Calu-3 monolayer - 3 μm pores

Carboxyl-modified particles

Amine-modified particles

Passage of 6% of carboxyl- and amine-modified particles
Results: Translocation studies

Calu-3 monolayer - 3 μm pore size

carboxyl-modified  amine-modified
Conclusions

- **TEER**
  - A549: no tight monolayer
  - Primary Type II pneumocytes: tight monolayer on 0.4 µm pores, but not on 3 µm pores
  - Calu-3: tight monolayer on 0.4 µm and 3 µm pores

- **Good correlation between TEER and sodium fluorescein leakage** ➔ minimal TEER

- **Translocation studies: 3 µm pores**
  - Calu-3: 6% translocation of 46 nm polystyrene particles
Thank you

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