



Development of an in vitro model for pulmonary translocation of nanoparticles

Jorina Geys

K.U.Leuven, Unit for Lung Toxicology



Introduction

- Nanoparticles = particle with at least one dimension $< 100\text{nm}$
- Nanotechnology & ambient ultrafine particles
- Epidemiological studies:
 - association between particulate air pollution and respiratory & cardiovascular mortality / morbidity



Introduction

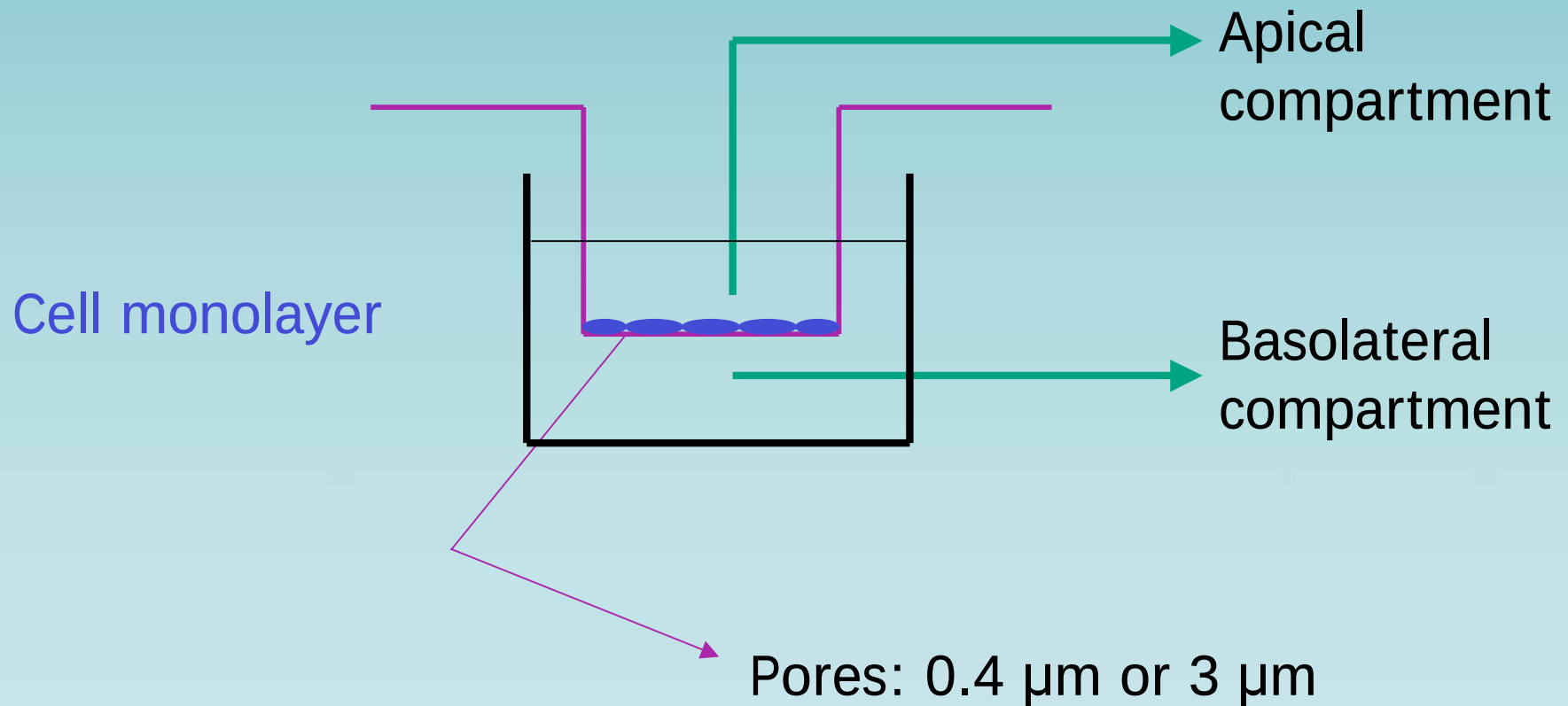
- In vivo experiments: translocation to the blood circulation (A. Nemmar, et al. 2001, 2002)
- In vitro: ?

➡ In vitro pulmonary translocation model

screen particles for translocation
what is the mechanism

Cell culture model

Transwell clear insert



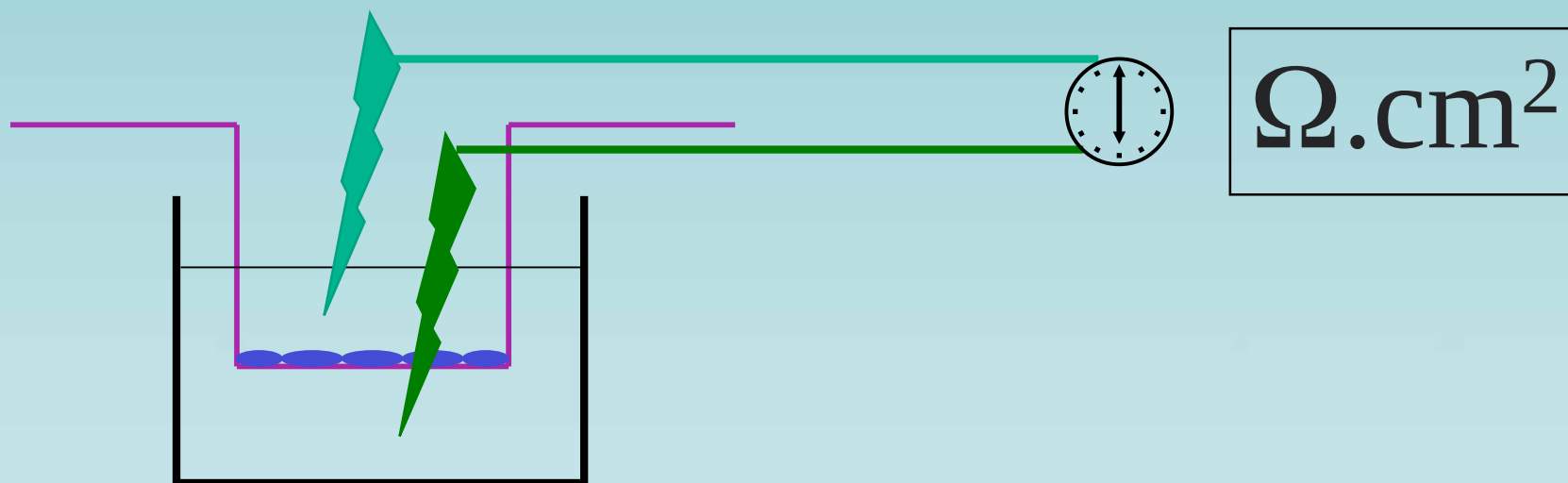


Materials & Methods

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

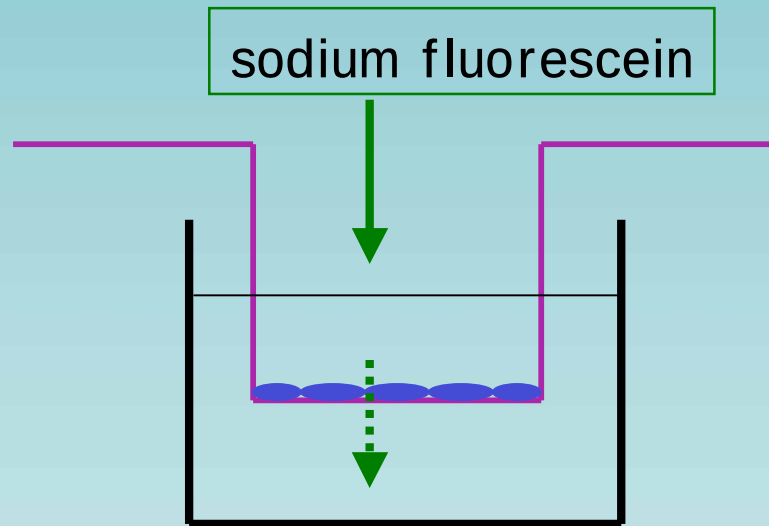
Materials & Methods

- Control integrity of cell 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

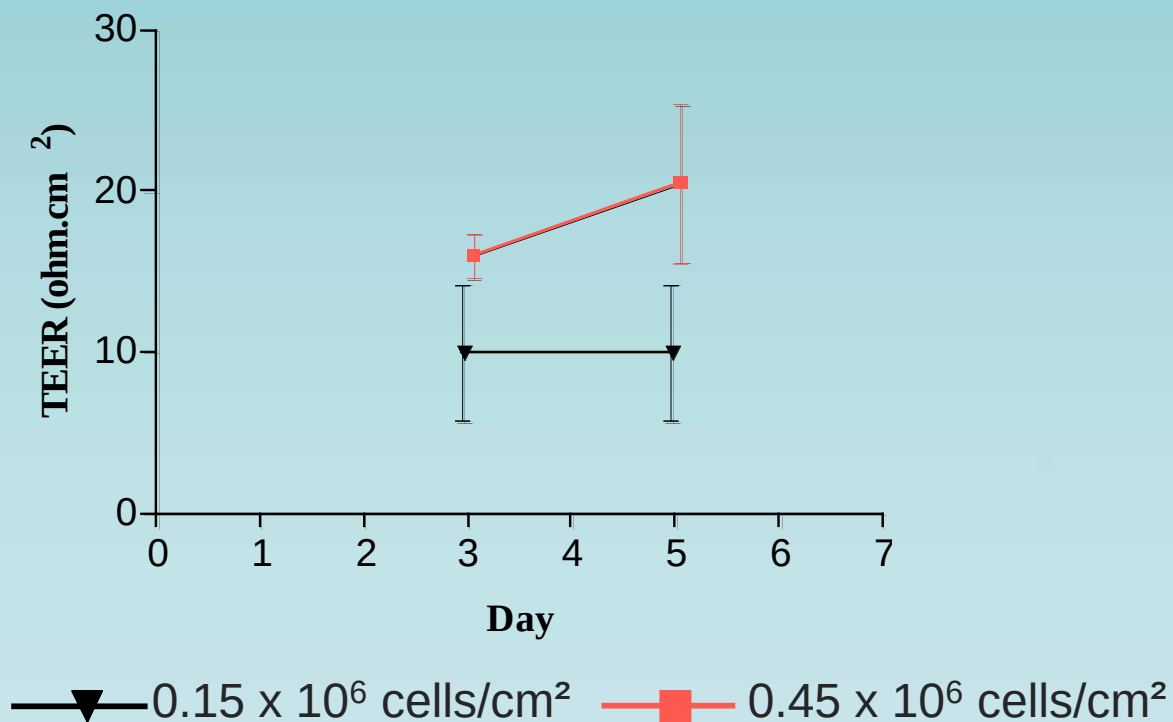


Materials & Methods

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

Results

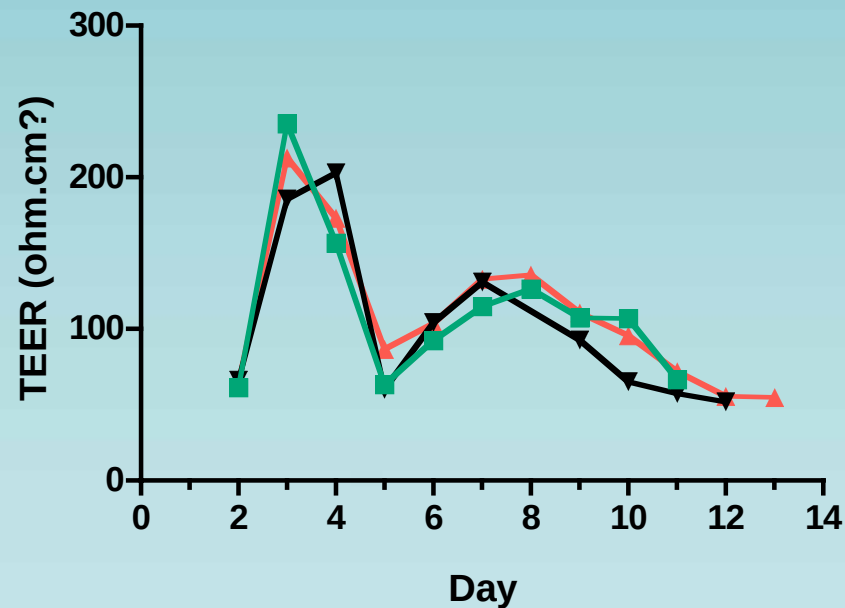
- Integrity of monolayer: TEER
 - A549 cell line -0.4 μm pores
 - Waymouth medium



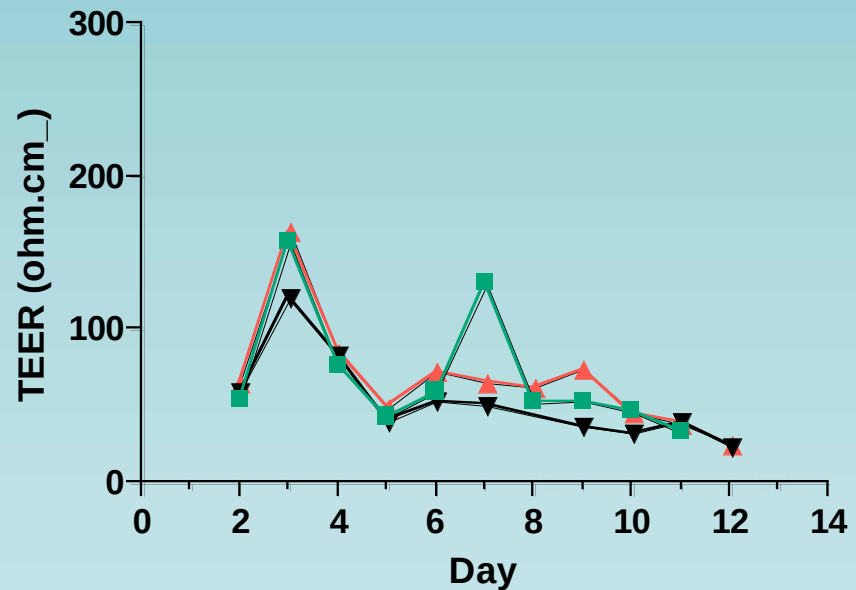
Results: TEER

- Primary Type II pneumocytes -0.4 μm pores
Waymouth medium

10% serum



2% serum



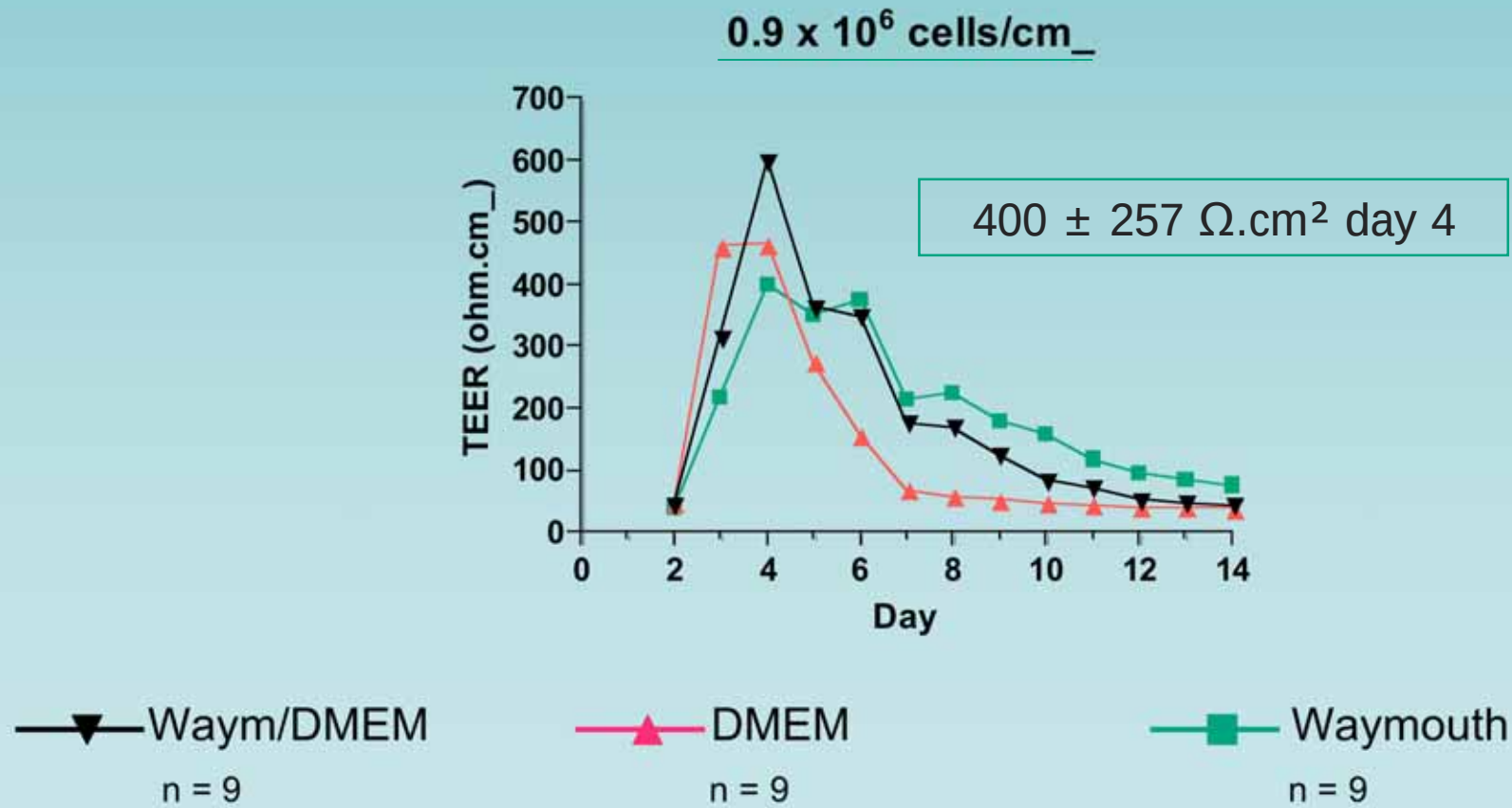
—▼— 0.3 x 10⁶ cells/cm²
n = 8

—▲— 0.45 x 10⁶ cells/cm²
n = 5

—■— 0.6 x 10⁶ cells/cm²
n = 6

Results: TEER

Type II pneumocytes -0.4 μm pores





Results: TEER

- Primary Type II pneumocytes – 3 μm pores
Waymouth medium

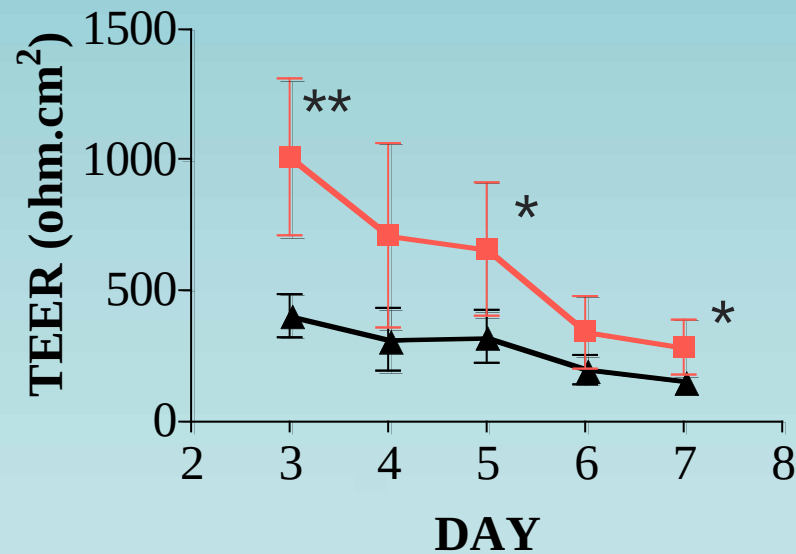
	Day 3	Day 5
0.6×10^6 cells/cm²		
No coating	$7 \pm 7 \Omega\cdot\text{cm}^2$	$31 \pm 13 \Omega\cdot\text{cm}^2$
Coating [#]	$10 \pm 7 \Omega\cdot\text{cm}^2$	$25 \pm 10 \Omega\cdot\text{cm}^2$
0.9×10^6 cells/cm²		
No coating	$8 \pm 3 \Omega\cdot\text{cm}^2$	$24 \pm 9 \Omega\cdot\text{cm}^2$
Coating [#]	$13 \pm 4 \Omega\cdot\text{cm}^2$	$30 \pm 6 \Omega\cdot\text{cm}^2$

mono-fibrillar collagen coating

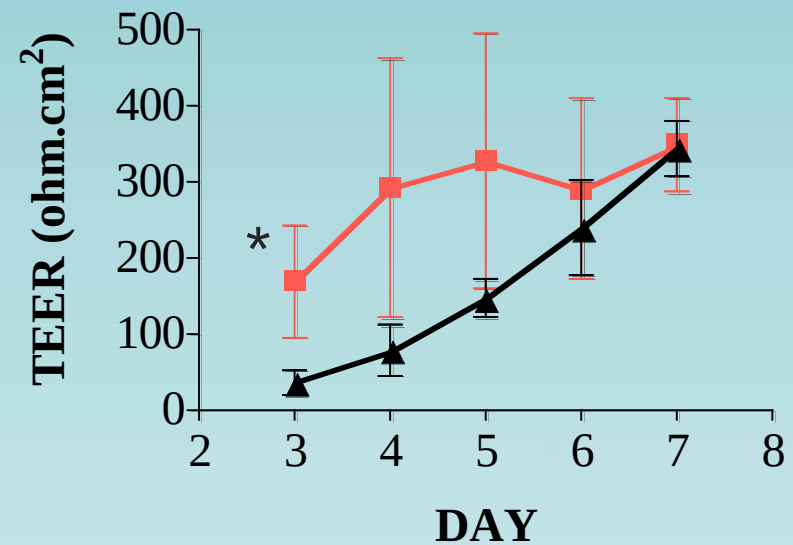
Results: TEER

■ Calu-3 cell line: MEM medium

0.4 μm pore size



3 μm pore size



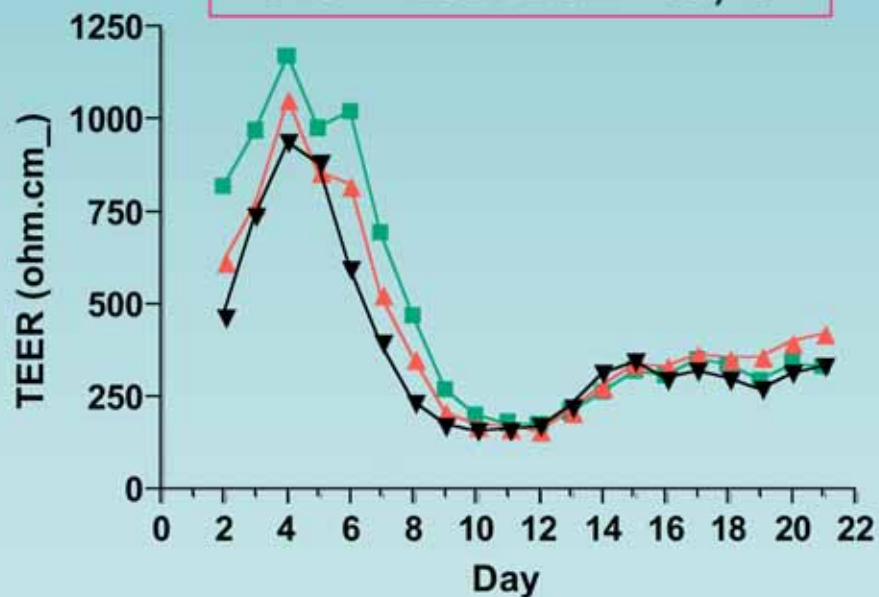
—■— 0.3 x 10⁶ cells/cm²
—▲— 0.6 x 10⁶ cells/cm²

Results: TEER

Calu-3: DMEM medium

0.4 μm pore size

$949 \pm 182 \Omega.\text{cm}^2$ day 3



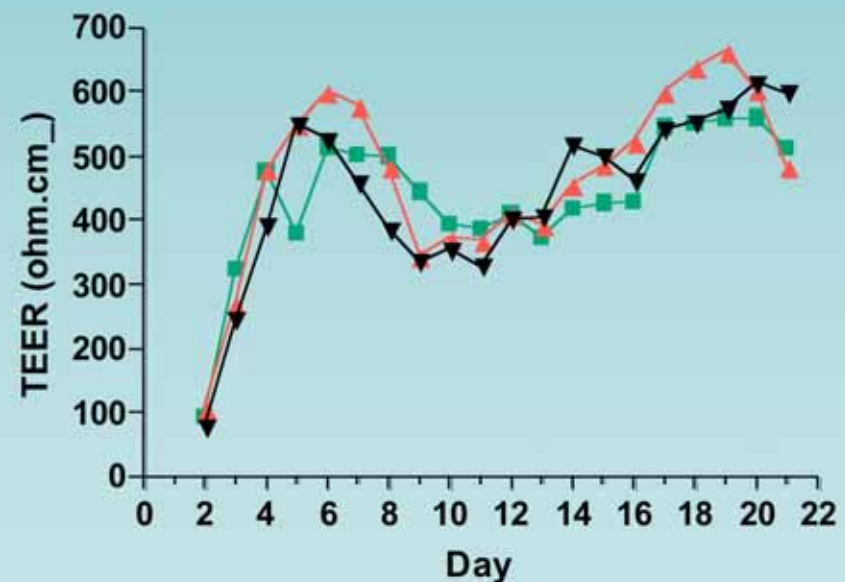
—▼— 0.6×10^6 cells/cm²
n = 4

—▲— 0.75×10^6 cells/cm²
n = 4

—■— 0.9×10^6 cells/cm²
n = 4

3 μm pore size

$500 \pm 95 \Omega.\text{cm}^2$ day 5





Results

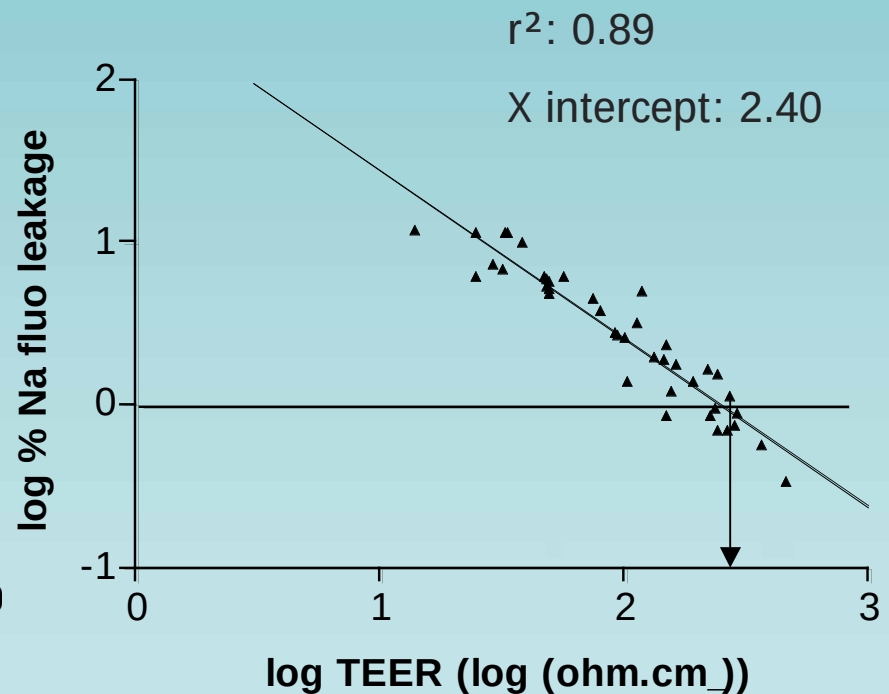
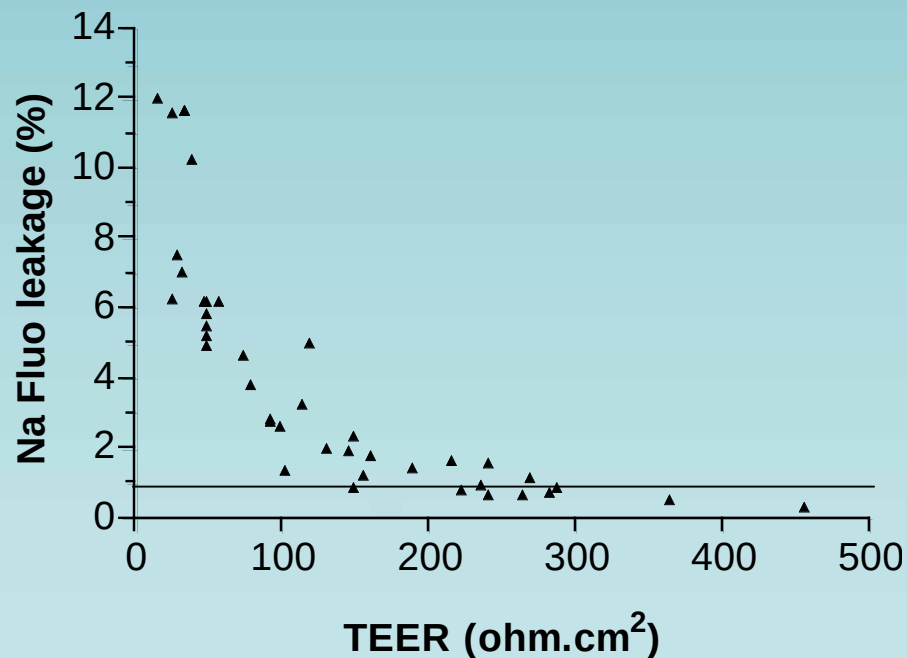
- Sodium fluorescein leakage

? Relation between TEER and
sodium fluorescein leakage

Relation between TEER and sodium fluorescein leakage

■ Primary Type II pneumocytes

0.4 μm pore size



minimal TEER: 251 $\Omega\text{.cm}^2$



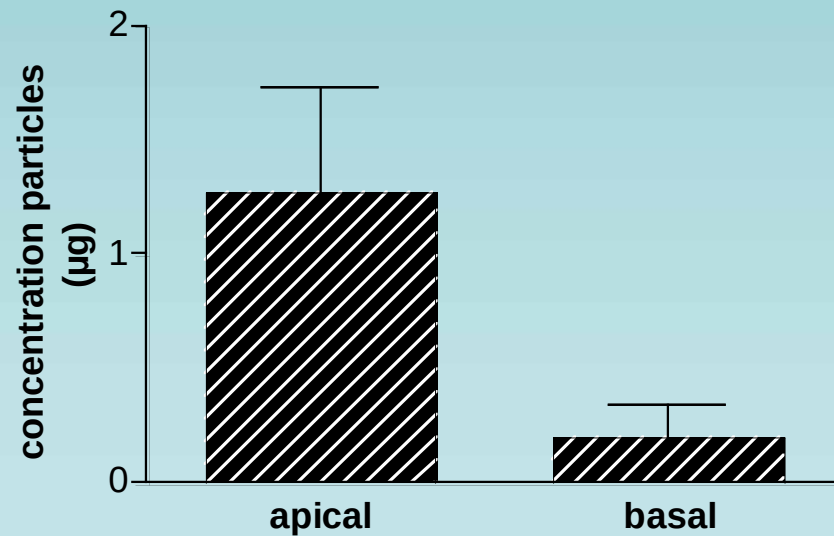
Relation between TEER and sodium fluorescein leakage

- Type II pneumocytes
 - 0.4 μm : $\sim 251 \Omega.\text{cm}^2$
- Calu- 3
 - 0.4 μm : $\sim 250 \Omega.\text{cm}^2$
 - 3 μm : $\sim 420 \Omega.\text{cm}^2$

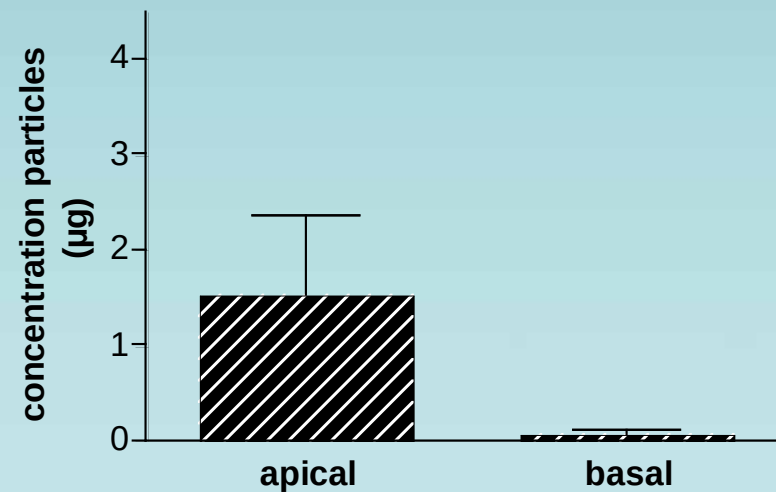
Results: Translocation studies

- Control inserts WITHOUT cell monolayer
 - 0.4 μm pore size

Carboxyl modified particles



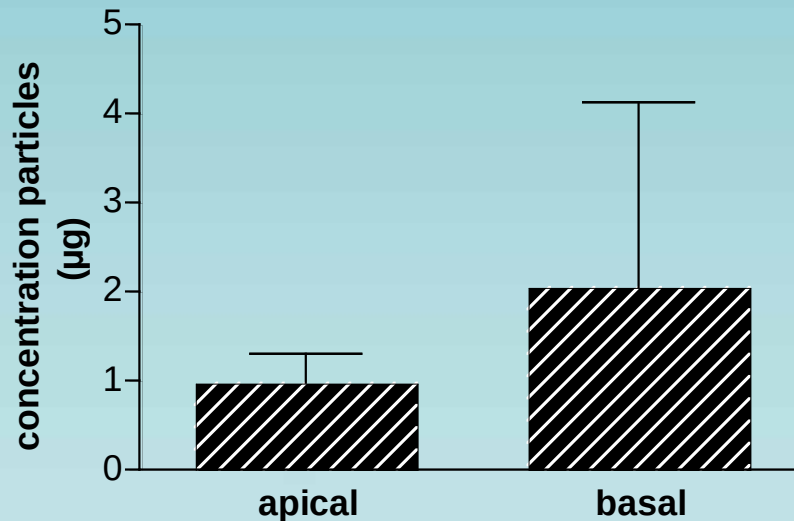
Amine modified particles



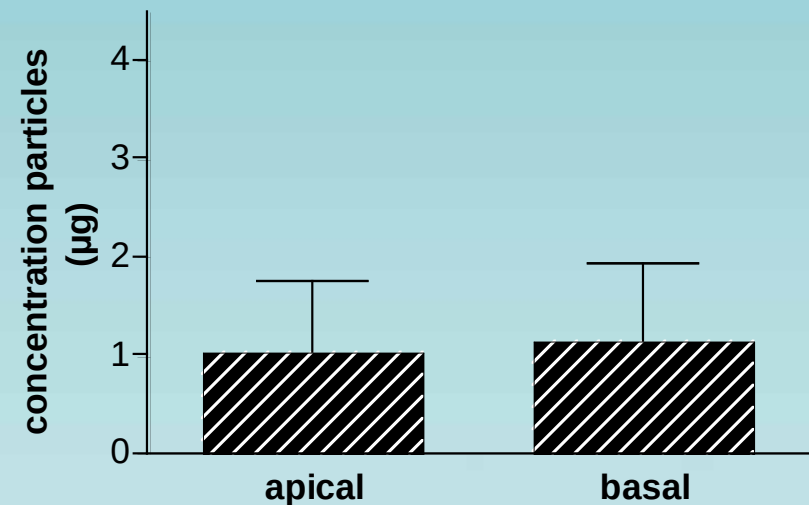
Results: Translocation studies

- Control inserts WITHOUT cell monolayer
 - 3 μm pore size

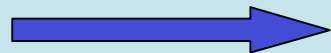
Carboxyl modified particles



Amine modified particles



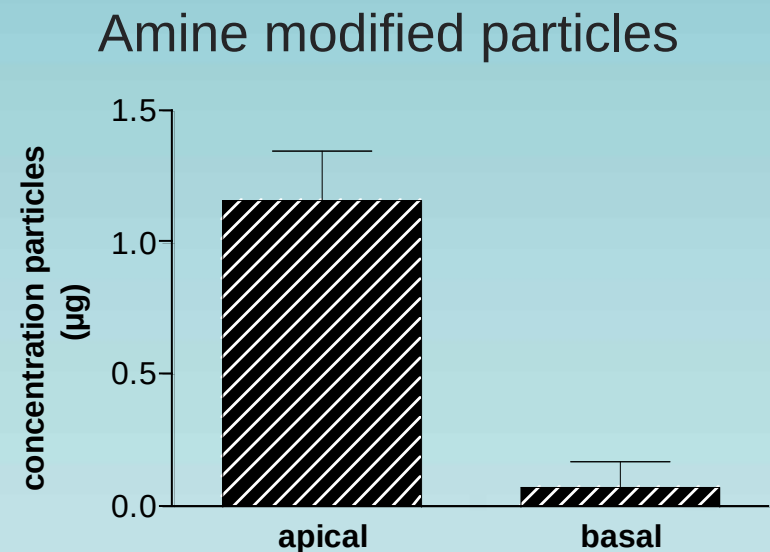
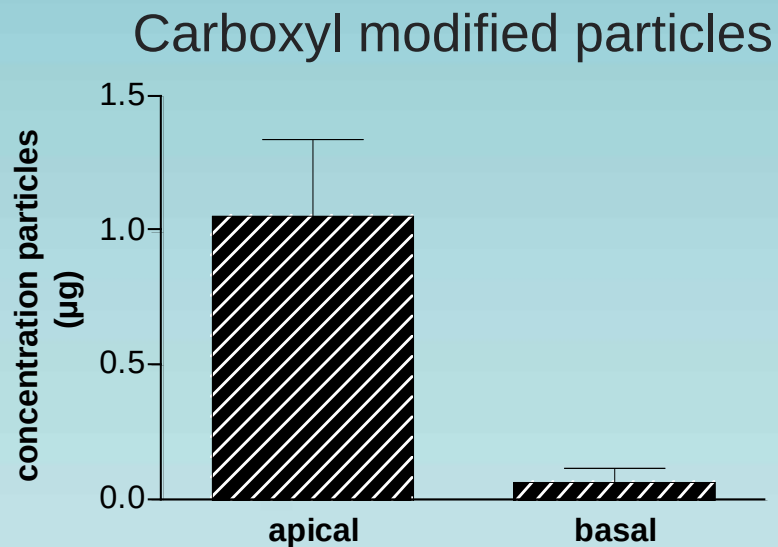
0.4 μm : passage of very small amount



3 μm : passage of 50%-80% of particles

Results: Translocation studies

- In presence of CELL MONOLAYER
 - Calu-3 monolayer - 3 μm pores



➡ passage of 6% of carboxyl and amine modified particles



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 $\longrightarrow$ minimal TEER
- Translocation studies: 3 μm pores
 - Calu-3 cell line: 6% translocation of 46 nm polystyrene particles



Thank you

Jorina.Geys@med.kuleuven.be

Geys, J., et al. 2006. In vitro study of the pulmonary translocation of nanoparticles. A preliminary study. Toxicol. Lett. 160, 218-226.