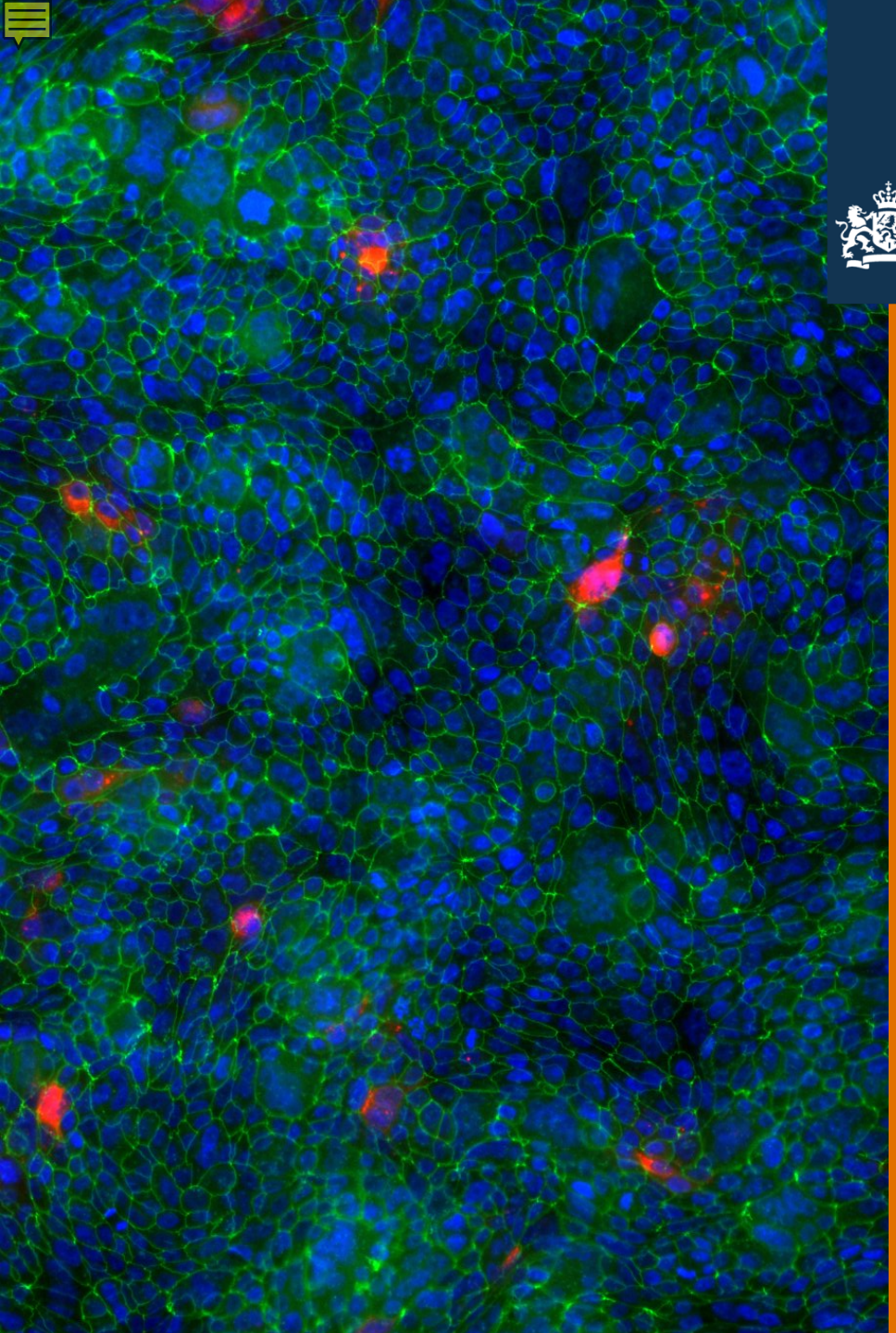


National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Optimization of an air-liquid interface (ALI) cell co-culture model to estimate the hazard of repeated aerosol exposures

Ruiwen HE (RIVM, the Netherlands)

nanoSAFE'20

23-11-2020

Content

- Optimization of an epithelial + macrophage co-culture model under ALI conditions

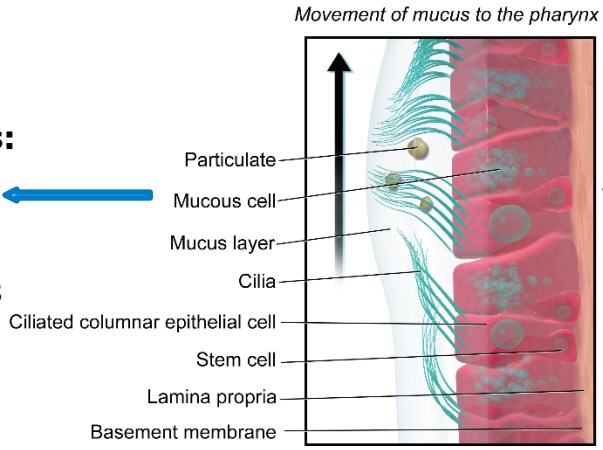
- Application of the co-culture model for ALI exposure to nanoparticles

Inhalation exposure to aerosols

There is an increasing request to use in vitro models for toxicity testing.

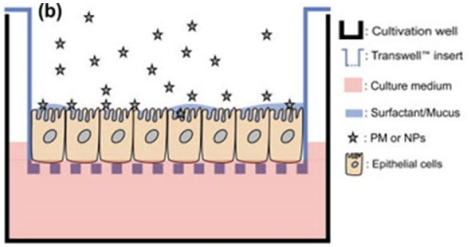
To closely mimic the inhalation exposure, **ALI cell culture and ALI exposure** were developed.

Cell models:
Calu-3
16HBE14o-
BEAS-2B
Primary cells
….



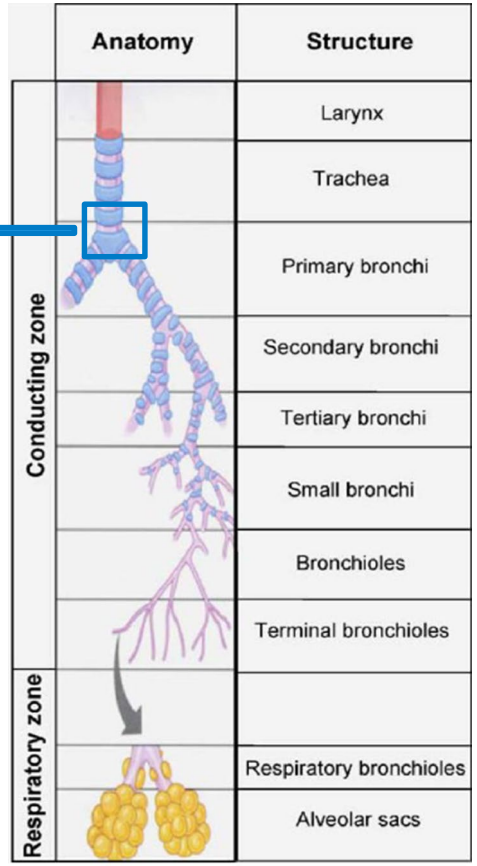
Lung bronchial epithelium

ALI culture



ALI exposure

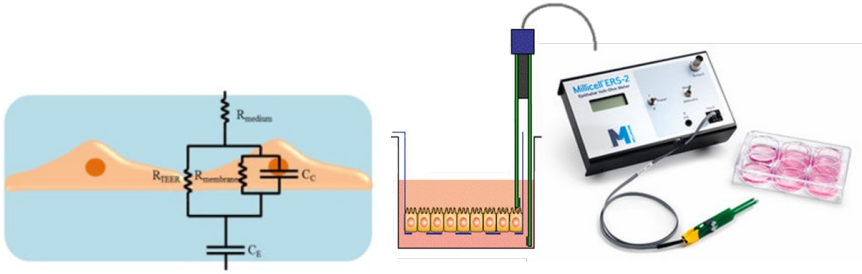
However, not many cell types can remain viable for long-term ALI culture, careful **selection of lung cell models** is needed.



Structures of the respiratory tract (BéruBé et al 2010)

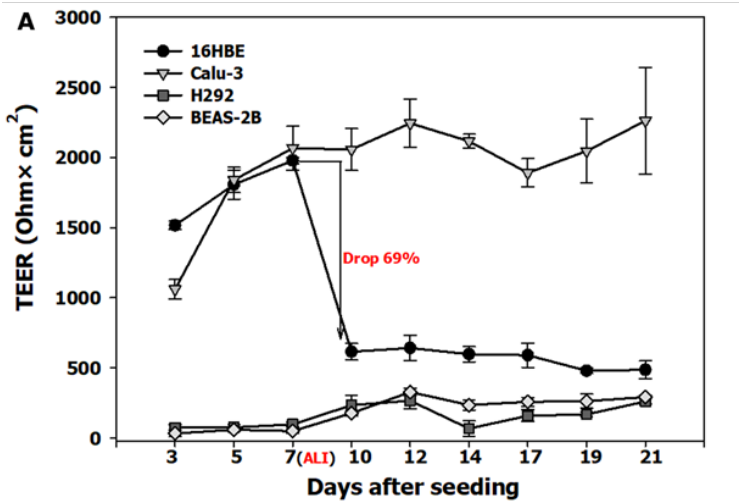
Epithelial cell models: Calu-3, 16HBE, H292, and BEAS-2B cell lines

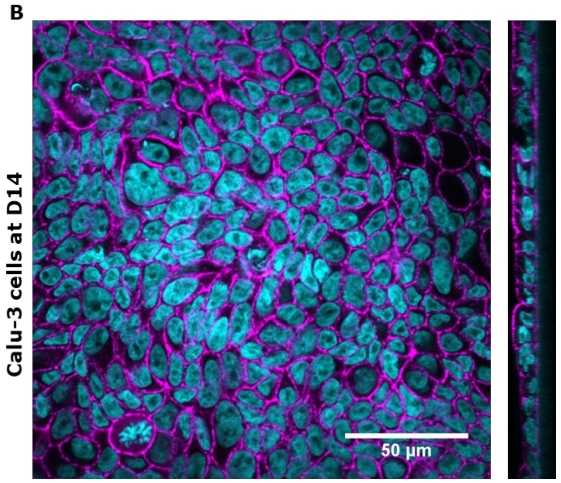
Epithelial barrier: barrier function and single-layer morphology are essential criteria



TEER measurement

Protein ZO-1 Staining



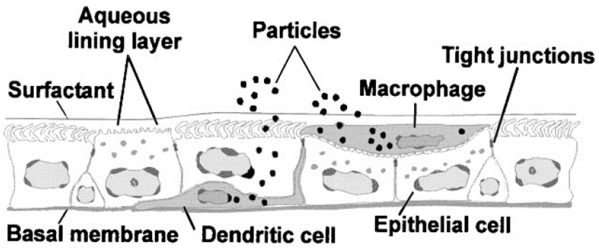


Only the **Calu-3 cells** can maintain the monolayer structure and a strong tight junction under long-term ALI culture.

TEER (A) and confocal fluorescence microscopy images (B) of Calu-3 cells at the ALI (He et al. 2020).

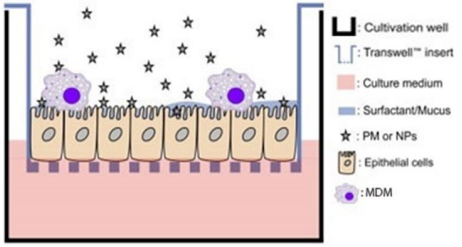
Epithelial cell + Macrophages co-culture

Epithelial Calu-3 cells:
1) Epithelial barrier
2) Platform for activation of immune cells
e.g. Macrophages in response to particles

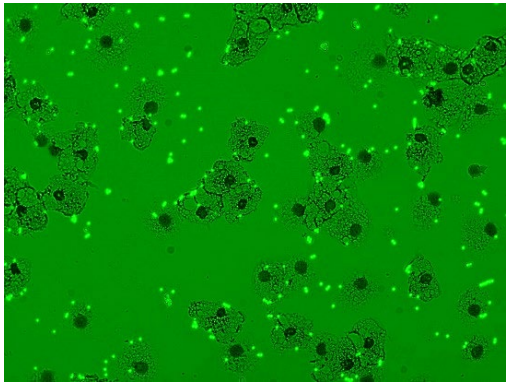


Airway wall–particle interaction (Rothen-Rutishauser et al. 2005)

To evaluate the realistic responses to particles, **co-culture models** need to be created by adding macrophages on the top of the epithelial cell layer.

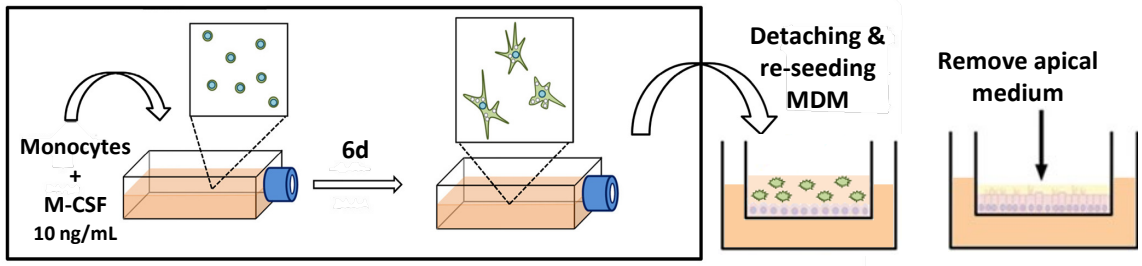


Co-culture model


Macrophages engulf the fluorescent particles

Building Calu-3 + MDMs co-culture model:

1. Differentiated macrophages are added onto Calu-3 cells layer (7d ALI culture)
2. After macrophages adhesion time, removing apical medium

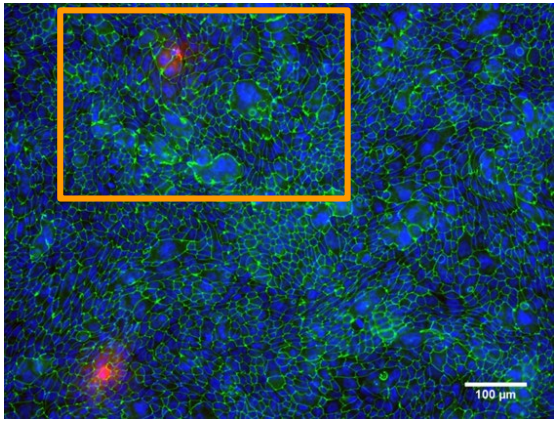


Optimization of Calu-3 + MDMs co-culture model at the ALI

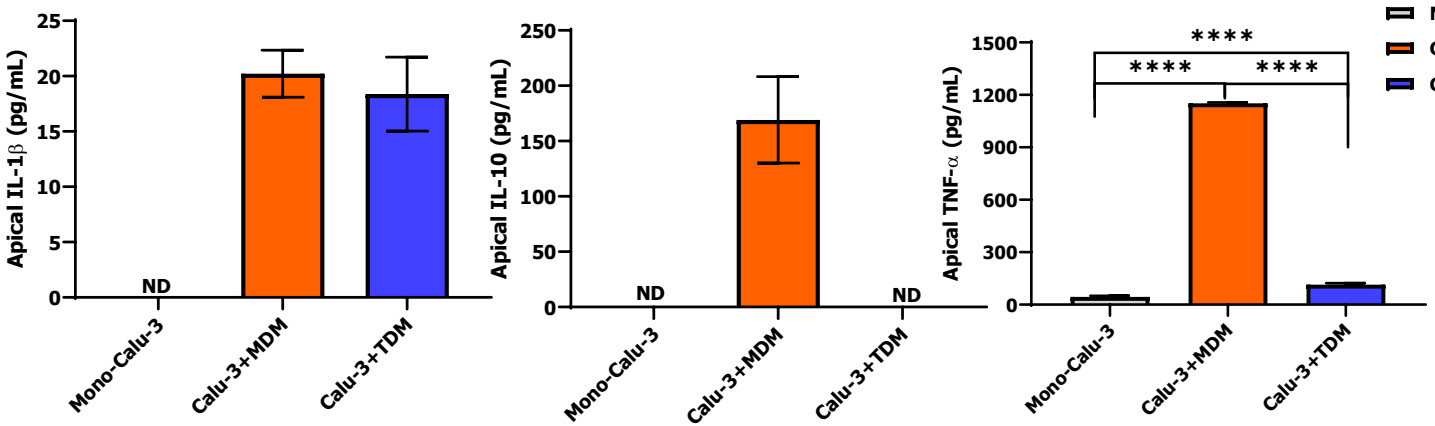
Macrophage adherent time and seeding concentration can affect monolayer structure and sensitivity of co-culture models.

With the optimization
Seeding density: 5×10^4 macrophages/$cm^{2.}$
Adhesion period of macrophages: 4 hours

Mutual comparison between Calu-3 mono-culture, Calu-3 + MDM, and Calu-3 + TDM models in response to LPS (He et al. 2020).



Multilayers after macrophages adhesion for 24 hrs (He et al. 2020).



MDMs:
Human monocyte-derived macrophages

TDMs:
THP-1 derived macrophages

Calu-3 + MDM model showed higher sensitivity in inflammatory responses to LPS exposure.

Application of Calu-3 + MDMs model

Single day exposure to nanoparticles (DQ12) :

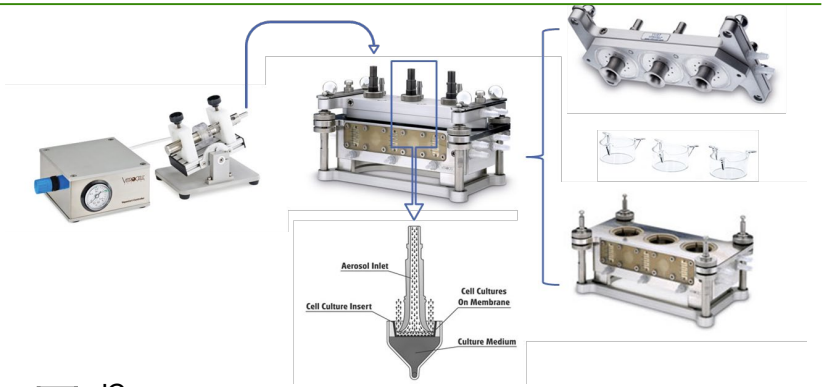
Continues flow ALI exposure system

Airflow: 50 ml/min

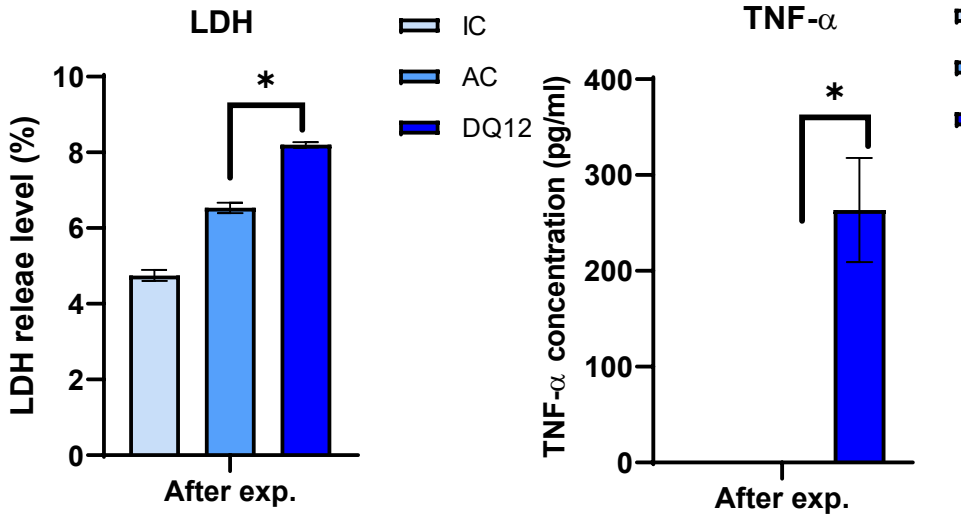
Exposure time: 4 hrs

DQ12 concentration: 1% DQ12 stock solution

Deposited mass: ≈ 2 µg/cm^2 (similar to an *in vivo* level)



ALI exposure system



- Calu-3 + MDMs co-cultures showed the inflammatory response (TNF-α) to DQ12 exposure.
- The repeated exposure experiments to DQ12 and other nanoparticles are ongoing.

Conclusions

- Calu-3 + MDM co-culture model had the epithelial monolayer integrity under long-term ALI culture
- Calu-3 + MDM co-culture showed the increased sensitivity in response to LPS and DQ12 exposure
- Calu-3 + MDM co-culture model is a preferred option for ALI exposure to aerosols for toxicity testing.