# PATROLS

Advanced Tools for NanoSafety Testing

# Lung cell models for short and long term engineered nanomaterial exposure at an air-liquid interface

- Adapted existing mono- and multi-cellular 3D systems mimicking the specific cell types in different lung compartments (Figure 1).
- Models have been developed to evaluate the safety aspect of engineered nanomaterial (ENMs) using short and long-term exposure approaches.
- Cells were grown in submerged conditions before switched to an air-liquid interface (ALI) allowing the exposure to an ENM aerosol.

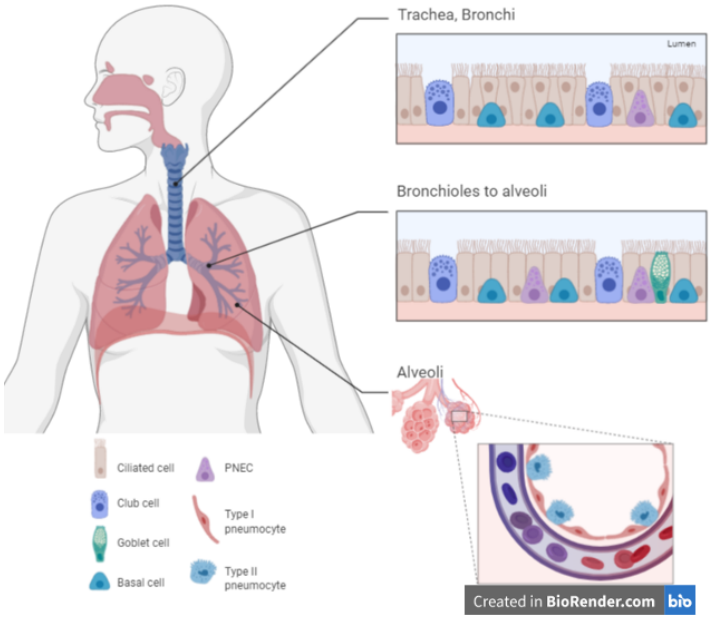



Figure 1.The pulmonary cells found within various regions of the lung. Made with BioRender.

## Bronchial region

### Calu-3 (ATCC® HTB-55™)

- Maintained tight junctions and a high trans-epithelial electrical resistance (TEER) when switched to the ALI.
- Maintained a stable monolayer for and can be cultured for up to 6 weeks at the ALI.

## Alveolar region

### hAELVI cell line (Inscreenex)

- Suitable for long term exposures
- Long growth time, but continuously stable
- Developed tight junctions 14 days in submerged conditions
- Maintained a stable monolayer for 14 days at an ALI

### A549 cells (ATCC® CCL-185TM)

- Not suitable for long term exposures at ALI
- Suitable for a maximum of 72 hour exposures (Figure 2)
- Cheap and easy to use and grow

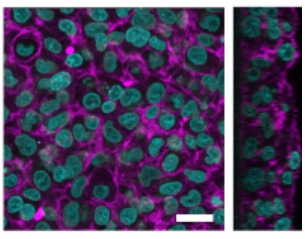


Figure 2: Characterisation of A549 cell layer cultured for 5 days in total (i.e. 4 days under submerged conditions followed by 24 hours at ALI). Scale bar: 20µm Blue = nucleus Magenta = cytoskeleton



This project has received €12.7M in funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 760813

## Co-Cultures (addition of macrophages)

- Addition of macrophages onto the apical side of the membrane
- Providing a relevant epithelial cell : macrophage ratio
- Primary macrophages (isolated from whole blood and monocyte derived macrophages (differentiated THP-1 (dTHP-1) cell line (Figure 3.)).

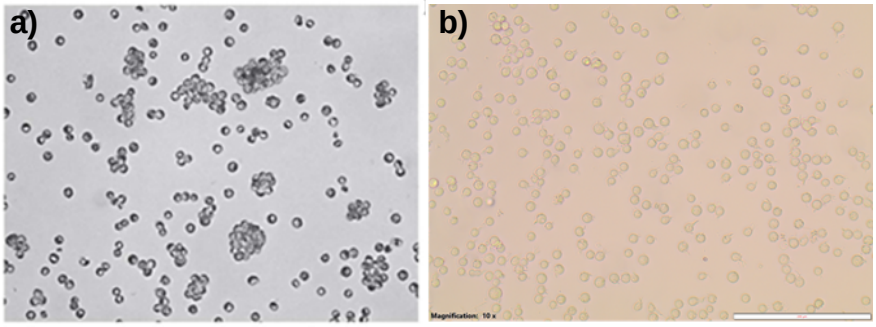
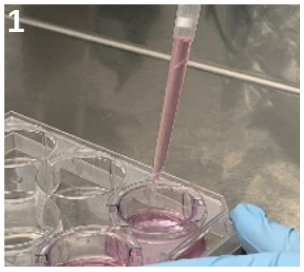


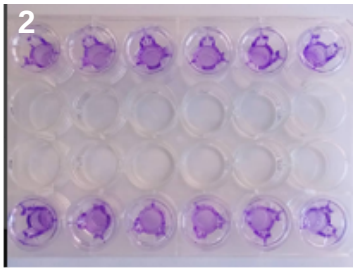

Figure 3. Images of (a) THP-1 cells in culture directly after seeding, (b) differentiated (dTHP-1) macrophages 48hr after seeding PMA.

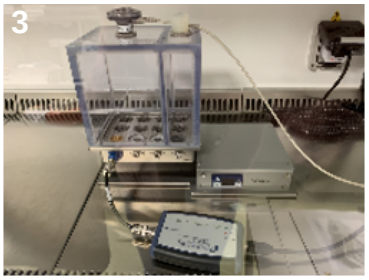
## ENM exposure methods

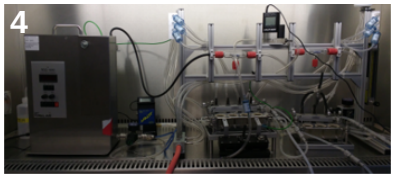
Depending on the available equipment the following exposure methods can be used:

1. Submerged exposures
   - Addition of a defined volume of ENMs suspension
2. Quasi-ALI exposures
   - Addition of small volume of ENM suspension covering the apical side of the cells.
3. Liquid aerosol exposures
   - A liquid aerosol containing ENMs is exposed to the cells
4. Dry powder exposures
   - A dry powder is exposed to the cells




## Conclusions

It is important to select the optimal cell line for the region of the lung of interest as well as the duration of exposures that are required. Each model has both pros and cons and these should also be considered.



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