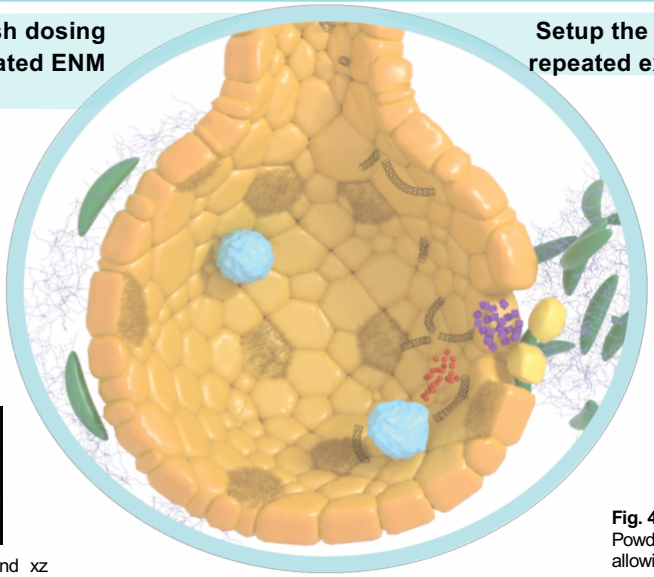
Predictive 3D lung models to assess the long-term hazard of nanomaterial aerosols *in vitro*

Hana Barosova[1]*, Kirsty Meldrum[2]*, Hedwig Braakhuis[3]*, Roberta Nossa[4], Rob Vandebriel[3], Arti Ahluwalia[4], Martin J. D. Clift[2], Barbara Rothen-Rutishauser[1]

Introduction: The lung is the primary route of entry for exposure to engineered nanomaterial (ENM) aerosols, thus the development of relevant and reliable models mimicking ENM inhalation is important. Predominantly, previous research has focused upon short-term, high-dose exposures using 2D monocultures. However, these model systems have limitations and therefore do not adequately represent the realistic human physiological environment, nor the responses following long-term ENM exposures.

Optimise lung models and establish dosing strategy to enable long-term, repeated ENM exposures;



Interlaboratory comparison of A549 cell line (human adenocarcinoma alveolar epithelial type II-like cells) was performed, and results show:

- Low cell layer integrity.
- Monolayer stability for up to 3 days at air-liquid interface conditions (ALI, **Fig. 1**).

=> *Cells are suitable for* ***acute*** (up to 72 hours) *exposures.*

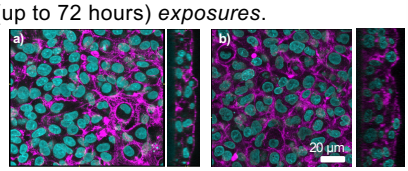


Fig.1: Laser scanning microscopy images (xy and xz projections) of A549 cells grown for **a)** 7 days under submerged conditions, **b)** 4 days submerged and 3 days at ALI. Magenta represents cytoskeleton, cyan cell nuclei.

Two other cell lines were identified for stable ***long-term*** exposures:

- **Calu-3** (human lung adenocarcinoma cells of bronchial epithelium, **Fig. 2a**).
- **hAELVi** (human lung alveolar epithelial cells type I-like, **Fig. 2b**).

Both cell lines showed:

- Tight cellular layer – high barrier integrity, expression of tight junction proteins.
- Stable monolayer over several weeks at ALI

=> *cells are suitable for* ***repeated, long-term*** *(*up to 3 weeks) *exposures.*

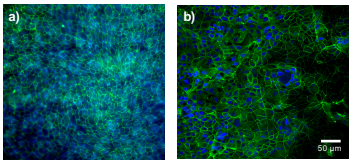


Fig.2: Laser scanning microscopy images of **a)** Calu-3 cells **b)** hAELVi cells cultured at ALI. Magenta represents cytoskeleton, cyan/blue cell nuclei, green tight junctions (ZO-1).

PATROLS Consortium has identified several well characterized ENM for exposures to be able to compare the data with *in vivo* results. Specifically, $BaSO_4$, crystalline Dörentruper Quartz (DQ_{12}) and Mitsui-7 multi-walled carbon nanotubes (MWCNT-7) were aerosolized (**Fig. 3**) using VITROCELL® Cloud system allowing low doses deposition at ALI (**Fig. 4**).

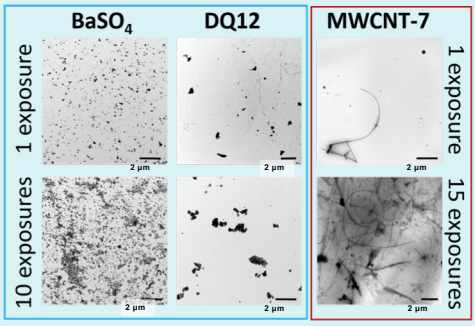


Fig. 3: TEM micrographs of particle deposited upon one and repeated aerosolizations of DQ_{12}, BaSO4, and MWCNT-7.

Setup the exposure protocol for long-term repeated exposures of ENMs at low doses;

Commercially available ALI exposure chambers: VITROCELL® Cloud system (**Fig. 4a**), VITROCELL® Powder Chamber (**Fig. 4b**) and VITROCELL® Automated Exposure Station (**Fig. 4c**) are being used within PATROLs experiments.

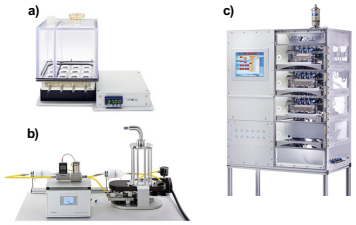


Fig. 4: VITROCELL® Exposure chambers a) Cloud, b) Powder Chamber, c) Automated Exposure Station allowing for repeated exposures of low doses. Source: www.vitrocell.com.

DALI (dynamic ALI) **system** (**Fig. 5**) is being developed within PATROLS **to simulate realistic breathing conditions,** and is composed of :

- Bioreactor with an **air-liquid interface.**
- **Stretching membrane** for cells seeding -> to mimic the breathing.
- Peristaltic Pump → **medium flow**.
- Aerosol generator.
- QCM → **to quantify** the online **particle deposition**.
- Control box with pressure regulators → to regulate the membrane stretching and mimic different stretching conditions.

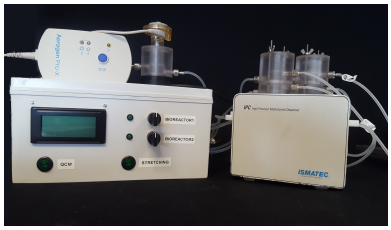


Fig. 5: DALI system, developed at University of Pisa, IT.

Conclusions:

- Advanced *in vitro* models of the human lung epithelial tissue barrier support improved ENM hazard assessment as physiologically relevant test systems that facilitate realistic exposure approaches to understand the impact of inhaled ENM.
- The thorough characterization of lung epithelial cell line growth as well as the optimization of ENM aerosolisation and dosing strategy are crucial for long-term experiments.
- We have identified suitable human lung epithelial cells representing the alveolar and bronchial compartment for long-term *in vitro* exposures. In addition, simulating the cyclic stretching in the lung, a new physiologically relevant bioreactor has been developed to mimic the dynamic micro-environment so as to improve the predictive power of exposure experiments.

***authors contributed equally**
[1] *BioNanomaterials, Adolphe Merkle Institute, University of Fribourg, Switzerland.*
[2] *In Vitro Toxicology Group, Swansea University Medical School, Wales, UK.*
[3] *Centre for Health Protection, RIVM, The Netherlands.*
[4] *Centro E Piaggio Research Centre, Faculty of Engineering, University of Pisa, Italy.*

@PATROLS_H2020
https://www.patrols-h2020.eu
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 760813

Presenting author: Kirsty Meldrum, Swansea University, email: kirsty.meldrum@swansea.ac.uk