PATROLS
Advanced Tools for NanoSafety Testing

3D lung cells to assess (long-term) effects of nanomaterials – Facts and recommendations

Background

The effects of long-term, repeated exposure to nanomaterials are unknown. Moreover, there are no valid assays to estimate the effects based on realistic exposure. Engineered Nanomaterials (ENM) have an increasing number of applications and the potential for ENM exposure of workers, but also consumers is increasing. Known toxicological tests are not representative for estimating possible adverse effects, such as fibrosis and cancer.

Pre-validation study

To evaluate potential adverse effects in human lungs after long-term, repeated exposure to ENM, PATROLS developed lung models to enable realistic ENM exposure. As a first step towards possible regulatory acceptance of these models, two of them were subjected to a pre-validation study. Such a study evaluates transferability (how well can a standard operating procedure (SOP) be transferred from one laboratory to another) and interlaboratory reproducibility (how well do the results between different laboratories compare).

The pre-validation study for the lung models consists of two parts

1. **Amount of ENM in contact with the cells**. A cloud of particles in the air is being made using a commercial system (Vitrocell® Cloud). The amount of particles from that cloud that land onto the cells is measured by weighing.
2. **Adverse effects in a lung model**. The lung models consist of human bronchial epithelial cells (i.e. Calu-3 cell line) cultured at the air-liquid interface. To mimic the physiology of the lungs more closely, macrophages, a type of immune cell, are added. These cells lay interspersed on the surface of the lower lung region. Next, these cells are important in the effect of ENM.

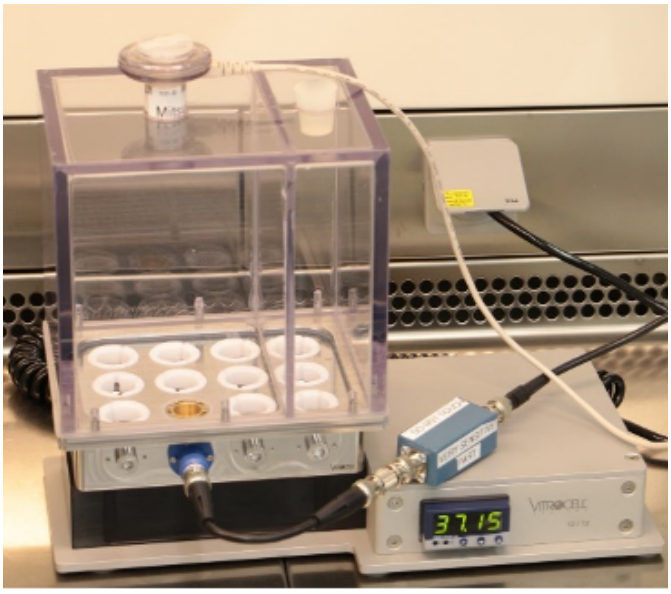

Figure 1. The Vitrocell ® Cloud system

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

Achievements

- These developments resulted in lung models with long-term, repeated and realistic ENM exposure with known deposition.
- Barrier function, cell viability and inflammatory mediators are measured
- We are currently executing an eight-partner interlaboratory comparison study, four within the PATROLS project and four outside the project.
- The transferability study resulted in SOPs, which will be made publicly available.
- Studies on the interlaboratory reproductibility are ongoing: the results will be published in 2021.

Lessons learned

1. **Efficiency of regulatory acceptance**: based on the experiences in PATROLS we can recommend that a pre-validation study first focuses on transferability, with SOPs being optimized, and then on inter-laboratory reproducibility.
2. **Education and capacity of trained personnel**: on-site training of personnel involved in an inter-laboratory comparison is important, but was, unfortunately, not possible due to the ongoing COVID situation. Support by e.g. webinars is advised so all partners acquire a similar level of knowledge.
3. **Avoidance of a European/international divide**: availability of the same consumables in different countries is a challenge. This might result in different experimental outcomes.
4. **Alternatives to animal testing**: for advanced *in vitro* models such as the ones in this study, SOPs need to be very detailed, in fact much more than we expected.
5. **Efficiency towards regulatory acceptance:** an ideal, but time- and resource-intensive scenario: (1) preparation of detailed SOP by the lead lab (SOP-1), (2) transfer to two other labs, resulting in an improved SOP (SOP-2), (3) inter-laboratory reproducibility study, possibly resulting in a further improved SOP (SOP-3), and (4) transferability to three naïve labs.

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