Bridging the Gaps in Nanosafety for Animal-Free Prediction of Adverse Outcomes

Vladimir Lobaskin [1], J. Štrancar[2], I. Urbančič[2], T. Stoeger[3], U. Vogel[4]

1. Introduction

On a daily basis, people are exposed to a multitude of health-hazardous airborne particulate matter with notable deposition in the fragile alveolar region of the lungs. Hence, there is a great need for identification and prediction of material-associated diseases, currently hindered due to the lack of in-depth understanding of causal relationships, in particular between acute exposures and chronic symptoms.

The current animal-based testing of novel materials' short- and long-term health effects is slow, expensive, and has limited capacity, which stifles the development of advanced materials and hinders efficient regulation of the market. To enable cost-efficient high throughput screening required for industry and regulation, one should shift the focus of nanosafety testing from late endpoints to early key events (KEs) leading to adverse outcomes (AOs). As such tests can only be based on mechanistic understanding, we need to close the knowledge gaps and match KEs *in vitro* and *in vivo*.

2. Methodology

Within the H2020 SmartNanoTox project, we had applied diverse advanced microscopies and omics to *in vitro* and *in vivo* systems, together with *in silico* molecular and coarse-grained modelling and determined herein that the long-lasting response to a single exposure can originate from the interplay between the newly discovered nanomaterial quarantining and nanomaterial cycling between different lung cell types [1]. This new insight allowed prediction of the spectrum of lung inflammatory responses associated with materials of interest using only *in vitro* measurements and *in silico* modelling, potentially relating outcomes to material properties for a large number of materials, and thus boosting safe-by-design-based material development.

Following the initial discoveries, in the Horizon Europe nanoPASS project we had developed the automated translation of the automated *in vitro* observations into *in silico* model, that enables automatic discovery of the early mode-of-action and further on automated disease evolution prediction. Because of its profound implications for animal-free predictive toxicology, this work paves the way to a more efficient and hazard-free introduction of numerous new advanced materials into our lives.

[1] University College Dublin, Dublin, Ireland; Vladimir.lobaskin@ucd.ie
[2] *Jozef Stefan institute, Ljubljana, Slovenia*
[3] *Helmholtz Zentrum München, München, Germany*
[4] *National Research Centre for the Working Environment, Copenhagen, Denmark*

Our key bridging methods are intravital in vivo microscopy, quantitative time-lapse *in vitro* microscopies, and automated identification of the modes of action (i.e. KE relationships) with proprietary *in silico* algorithms, supported by datamining of the worlds' largest *in vivo* database and single-cell omics data, and computational modelling of structure-function relationships.

3. Results

In the nanoPASS project, we aim to 1) develop new *in vitro* systems that can replicate early KEs leading to AOs related to inhalation of NMs, 2) identify methods to track the dynamics of these KEs, 3) develop quantitative *in silico* models to predict AOs, and 4) calibrate the *in vitro/in silico* AO predictions against *in vivo* data for 40+ well-characterised benchmark materials. We will also validate the AO predictions on several families of industrial materials, sampled from different stages of their life cycle, and then propose reliable testing protocols and guidelines. With the consortium of 6 complementary research laboratories, SME as technology developer and provider, material producing company as potential end-user, and an industrial association to facilitate dissemination, nanoPASS covers the whole value chain of the new animal-free safety testing technology, and thus paves the way towards safe adoption of new nanotechnologies.

4. Acknowledgements

We acknowledge funding from the EU Horizon2020 framework under grant agreements No. 686098 (SmartNanoTox project), Horizon Europe under grant agreement No. 101092741 (nanoPASS project).

5. References

1. Kokot, H. et al. 2020, *Prediction of Chronic Inflammation for Inhaled Particles: the Impact of Material Cycling and Quarantining in the Lung Epithelium*, Adv. Mater., 32, 2003913 (2020).