PATROLS
Advanced Tools for NanoSafety Testing

Robust lysosomal dissolution media

Challenge of pulmonary clearance

Dissolution of inhaled nanomaterials (ENM) under physiological conditions is essential to predict the clearance of the ENM from the lung, and to assess the potential effects of released ions. Here we focus on the alveolar macrophage (AM) lysosomal condition, where dissolution rates may be modulated by the acidic pH, salts, enzymes and other components. In a comprehensive literature review, the enzymes and proteins that comprise the lysosomal fluid are widely known -but not constant and not easily reproduced-, comprehensive ionic composition data remain unavailable.

Implementation of a robust alternative method

In practice, a variety of 13 different pH 4.5 fluids simulating AM lysosomes were used in literature, of which we selected six diverse fluids and tested their modulation of the dissolution kinetics, determined by the ions released in continous flow system, and the modulation of the ENM particle size, determined by a validated TEM image analysis. Three lysosomal simulant media were consistent with each other and with in vivo clearance. These media correctly predict both the quick dissolution of ZnO, the partial dissolution of SiO_2, and the very slow dissolution of TiO_2 .

Scientific reasoning

The choice of the organic acids (see figure) that mimic the enzymes of the in-vivo conditions is essential. The valid media either use a mix of organic acids (with total concentration below 0.5 g/L, thereof citric acid below 0.15 g/L) or another organic acid (KH phthalate) at 4 g/L. For several ENM, including ZnO, $BaSO_4$, CeO_2, all these differences induce only minor modulation of the dissolution rates. Only for Ti and Si ions, the sequestration by specific organic acids is highly sensitive, and can lead to wrong predictions when compared to in vivo behavior. The media that fail on TiO_2 and SiO_2 dissolution use citric acid at concentrations above 5 g/L (up to 28 g/L).

Recommendations for OECD guidance development

We recommend the standardisation of ENM dissolution testing by one of three valid lysosomal simulant fluids with determination of the dissolution rate and halftime by the quantification of ions. This recommendation was established on the continuous flow system but is expected to be relevant as well for static (batch) solubility testing. Both are currently standardised by an ongoing OECD project.

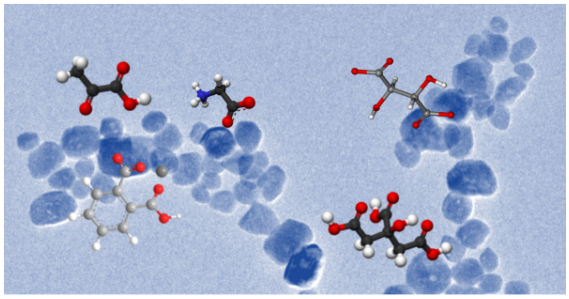

Figure 1. Organic acids constitute the different lysosomal simulant fluids that interact with the ions that are dissolved from nanomaterials (Organics and ENM are not to scale. The specific example is BaSO4 NM220, imaged after 1 week dissolution in one of the tested fluids).

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