Highlights from NanoMILE's recent publications: a progress update for the NanoSafety Cluster

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FP7 project NanoMILE (www.nanomile.eu) is a unique partnership focussed on developing detailed mechanistic understanding of the interactions of manufactured nanomaterials (MNMs) with living systems.

NanoMILE intends to revolutionise nanosafety research through its robust and novel approaches to the selection and development of test MNMs including environmentally aged variants to represent real-world materials, its technically and computationally advanced integration of systems biology, its thoughtfully balanced toxicological / ecotoxicological approaches, its development of novel high throughput platforms for screening and its feedback loops for development of MNMs that are safer by design.

Together, these approaches will result in a robust framework for classification of MNMs according to their biological impacts. Several important advances have already been made, in the first 18 months of the project, with some highlights presented below.

Towards a strategy for grouping and classification of Nanomaterials

Within NanoMILE, UoB and KIT developed a hypothesis that nanomaterial (NM) toxicity can be predicted as the sum of three "quantifiable" parameters (principle components, as shown in Figure 1) that capture the diversity of modes of action of NMs, namely:

- *Composition* which includes inherent molecular toxicity, charge, hydrophobicity and coating (although also linked to both the intrinsic and extrinsic axes).
- *Intrinsic* properties which are inherent to the nano-form of a material, and include e.g. structure and structural strain. A number of NM physicochemical properties map onto the *intrinsic* axis, including shape, porosity, structural configuration and bandgap.
- *Extrinsic* properties which are those corrected to the surface area of the NM, including e.g. surface interactions and transformations of NM surface and biomolecules (e.g. unfolding, receptor activation, membrane damage, fibrillation etc.) as a result of binding.

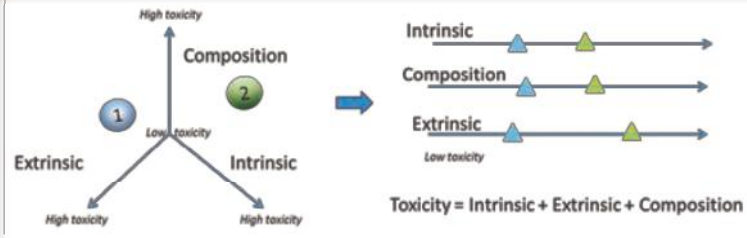

Figure 1: NanoMILE three principle axes that describe toxicity and mode of action.

Key features of this approach are that:

1) it allows separation of modes of action e.g. dissolution is primarily associated with specific NM *compositions*, but can be facilitated by specific *intrinsic* properties such as high strain conformation associated with, for example, pointed structures such as needles or nano-stars, and by *extrinsic* factors such as strongly binding ligands that unfold and expose cryptic epitopes for example; and
2) identities of the major physico-chemical descriptors driving the toxicity from the weight of contribution to each principle component/axes can be explicitly determined (i.e. the loadings), as can the main mode of action as this will be the principle component with accounting for the highest amount of the variability in the data. Thus, we envisage utilisation of a set of scales from low to high (toxicity) for each of the three parameters with the overall toxicity being the sum of the three axes (see Figure 1).

The fact that the same physico-chemical descriptor can contribute to more than one PC is a key feature of this approach, which we expect will enable development of QNARs, facilitate the grouping of NMs on the basis of where they sit in this 3-dimensional space, and support regulatory decision-making.

See Lynch, et al., **Nano Today,** 2014, 9:266. http://dx.doi.org/10.1016/j.nantod.2014.05.001.

Open access platform for modelling and prediction of nanomaterial uptake

In an effort to computationally explore available datasets that will lead to ready-to-use applications one of NanoMILE's SME partners (Novamechanics) has developed and validated a quantitative nanoparticle-activity relationship (QNAR) model for the prediction of the cellular uptake of nanoparticles in pancreatic cancer cells. Their insilico workflow was made available online through the Enalos InSilicoNano platform (http://enalos.insilicotox.com/QNAR_PaCa2/), a web service based solely on open source and freely available software that was developed with the purpose of making the model available to the interested user wishing to generate evidence on potential biological effects in the decision making framework.

This web service will facilitate the computer aided nanoparticle design as it can serve as a source of activity prediction for novel nano-structures. To demonstrate the usefulness of the web service the work exploited the whole PubChem database within a virtual screening framework and then used the Enalos InSilicoNano platform to identify novel potent nanoparticles from a prioritized list of compounds.

See Melagraki and Afantitis, ***RSC Adv.***, 2014, **4**:50713. http://pubs.rsc.org/en/content/articlelanding/2014/ra/c4ra07756c#!divAbstract

Cellular self-organisation and mobility on micro-structured surfaces: towards single cell HTS

Partner LMU has been working on methods to pave the way for automated filling of cell arrays, enabling high-throughput single-cell analysis of cell samples without losses. LMU reported recently on the phenomenon of cellular self-organization, which allows for autonomous positioning of cells on micro-patterned surfaces over time. This was facilitated by preparing surface with a regular lattice of protein-coated adhesion sites surrounded by polymer (PLL-g-PEG) passivated areas, which reduce protein biding and thus cellular adhesion, and studying the time course of cell ordering.

After seeding, cells randomly migrated over the passivated surface until they find and permanently attach to adhesion sites with occupancy levels typically reaching 40-60% after 3-5 h. The time required for sorting was found to increase with increasing distance between adhesion sites, and is well described by the time-to-capture in a random-search model. Further studies assessed cell behaviour (mobility) on polymer surfaces of different composition incubated with fibronectin (FN) using time-lapse microscopy, and correlated adhesion with the amount and location of FN via neutron reflectivity. Cells exhibited 21% increased motility on PLLPEG (5 kDa PEG chains) compared to pure FN layers, and 12% decreased motility for PLLPEG (2 kDa PEG chains), suggesting that by design of PEGylated surfaces cell migration can be controlled.

See Röttgermann et al., ***Soft Matter.*** 2014 Apr 14;10 (14):2397-404. doi: 10.1039/c3sm52419a and Röttgermann PJ et al., ***Macromol Biosci.*** 2014 Sep 10. doi: 10.1002/mabi.201400246.

Comparison of 5-day and 28-day inhalation toxicity responses to ceria nanomaterials

Two ceria nanomaterials (NM-211, NM-212) were tested for inhalation toxicity and organ burdens in order to design a chronic and carcinogenicity inhalation study (OECD TG No. 453). Rats inhaled aerosol concentrations of 0.5, 5, and 25 mg/m³ by whole-body exposure for 6 h/d on 5 consecutive days for 1 or 4 weeks with a post-exposure period of 24 or 129 days, respectively. Lungs were examined in bronchoalveolar lavage fluid (BALF) and histopathology.

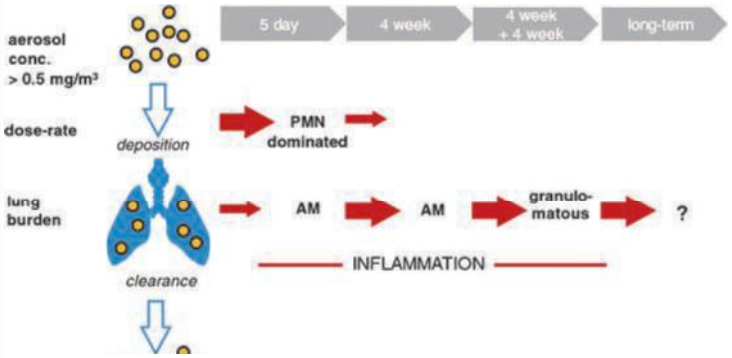

Figure 2: Summary of results after 5 days and 4 weeks of inhalation exposure to Ceria nanomaterials

Inhaled ceria NM-212 was deposited in the lung and cleared with a half-time of 40 days; at aerosol concentrations higher than 0.5 mg/m³, this clearance was impaired resulting in a half-time above 200 days (25 mg/m³). After 5 days of exposure to concentrations >0.5 mg/m³ ceria induced an early inflammatory reaction by increases of neutrophils in the lung which decreased after the exposure was terminated. The neutrophil number observed in bronchoalveolar lavage fluid BALF was also decreased after 4 weeks of exposure (compared to 1 weeks of exposure) and an increase of mononuclear cells, especially macrophages was observed. Macrophages were visible in histopathology but not in BALF. Further progression to granulomatous inflammation was observed after 4 weeks plus 4 weeks post-exposure (Figure 2). The surface area of the two ceria nanomaterials (NM-211, NM-212) deposited in the lung, rather than mass or volume, correlated with the inflammatory reaction after 1 week of exposure. Observing the time course of lung burden and inflammation, it appears that the dose rate of particle deposition drove an initial inflammatory reaction by neutrophils. The later phase (after 4 weeks) was dominated by mononuclear cells, especially macrophages. The progression toward the subsequent granulomatous reaction was driven by the duration and amount of the particles in the lung. The further progression of the biological response will be determined in the ongoing long-term study.

See also: Keller et al. ***Arch Toxicol.*** 2014 88:2033-59. http://www.ncbi.nlm.nih.gov/pubmed/25273020