Advanced *in vitro* liver and lung model development for engineered nanomaterial hazard assessment.

Samantha Llewellyn, Kirsty Meldrum, Gareth J. S. Jenkins, Martin J. D. Clift and Shareen H. Doak

In Vitro Toxicology Group, Swansea University Medical School, Swansea University, Singleton Campus, Centre for NanoHealth, Institute of Life Sciences, SA2 8PP, Swansea, Wales, UK

**Introduction:**
Exposure to engineered nanomaterials (ENM) poses a potential risk to human and environmental health through long-term, repetitive, low-dose exposures. Current ENM hazard assessment tools are based on short term, high-dose exposures using simple 2D *in vitro* test systems, which lack environmental realism in terms of dose delivery, exposure duration and biological complexity. Thus, there is an urgent need for more realistic and predictive *in vitro* test systems for ENM safety assessment. Physiologically Anchored Tools for Realistic nonOmaterial hazard aSsessment (PATROLS) seeks to overcome these disadvantages.

**PATROLS Aims to:**
Establish and standardise a battery of innovative, next generation hazard assessment tools that more accurately predict adverse effects caused by long-term (chronic), low dose ENM exposure (example Figure 1) in human and environmental systems to support regulatory risk decision making and help reduce the need for animal testing.

**Methods:**
- **Characterisation** will be completed during the growth and optimisation of the tissue models and after the addition of particles to the systems.
- **Cytotoxicity Analysis:** Viability count (Trypan Blue & Propidium Iodide/Hoescht).
- **Membrane Analysis:** Transepithelial electrical resistance (TEER).
- **Genotoxicity Analysis:** Cytokinesis Block Micronucleus (CBMN) assay.
- **Colormetric Analysis:** LDH/Lactate Assays.
- **Liver Function Analysis:** Albumin and Urea production.
- **Transwell Analysis:** Macrophage adherence, surface morphology.

**Liver Model**
- 3D HepG2 liver epithelial models formed using a hanging drop set up over 3 days before transfer into 96-well plates with agarose coating.
- HepG2 cells seeded at 4000 cells/well and remained viable for long term (>14 days) culture and thus is able to support extended and repeated ENM exposure.

**Liver Functionality**
- Liver model generated in 96-well plates with agarose-coated wells up to 10-14 days post-seeding.

**Genotoxicity**
- Micronuclei frequency (%): Comparison of (A) Acute (24hr) and (B) Long term (120hr) exposures to DQ Azure (A and D) and ENM suspension (C and F). N=3 subjects with all assays performed in triplicate. The data are presented as mean±SEM.

**Further development of the models**
Specific organ dynamics need to be taken into consideration, including movement (i.e. breathing motion) and flow dynamics (i.e. blood flow) via the use of 3D printing and cell types (such as immune cells) found in the in vivo system.

**Conclusion:**
Neither ENM, nor exposure scenario influenced the endpoints analysed. It is intended that following the successful development of such models, they can be used to establish advanced *in vitro* testing methods that will contribute towards the reduction of *in vivo* testing approaches across toxicology and drug discovery research.

**References:**
- https://www.patrols-h2020.eu
- https://www.swansea.ac.uk/library-how/heart-liver-work
- https://www.ebi.ac.uk/lung/respiratory-system