Augmented adverse effects of nanomaterials on diseased liver – implications for risk stratification?

Background

Meaningful *in vitro* safety assessment has numerous challenges with the overall aim of the generation of test systems which are able to mimic *in vivo* biological responses. As two important considerations, human test systems should overcome species-dependent limitations and uncertainties of animal-based *in vivo* risk assessments. Additionally, toxicity studies should not only be focused on the healthy population but also on susceptible individuals, e.g. people with medical conditions and the aged population.

The liver is the metabolic and detoxification centre of the body. A growing body of literature has shown that a proportion of nanomaterials (NM)s administered via inhalation and to a lesser extent following ingestion will translocate to a range of secondary organs including the liver. Importantly, with the clear advances in the field of nanomedicine there is the potential for intravenous and direct injection of NMs into the bloodstream. It is now understood that for foreign bodies reaching in the blood, the liver is key and the forefront to the xenobiotic challenge. Moreover, recent studies have demonstrated that responses from susceptible population to nanomaterial exposure are often more severe which is of particular importance for the liver.

Challenges

Our goal was to develop physiologically relevant hepatic human *in vitro* test systems which:

- Incorporated all relevant parenchymal (hepatocytes) and non-parenchymal (Kupffer cells, endothelial cells and stellate cells)
- Models that were representative of the healthy tissue as well as compromised liver reflecting the state of benign fatty liver or pre-fibrotic non-alcoholic steatohepatitis (NASH)
- Provide a test platform as ready-to-use, amenable to automated processing in a standard multi-well plate format.
- Systems that would allow chronic toxicity testing with repeated dosing, enabling the investigation of regeneration potential upon xenobiotic challenge which is of utmost importance for the liver as well offering read-outs relevant for mechanistic investigations.

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

PATROLS

Advanced Tools for NanoSafety Testing

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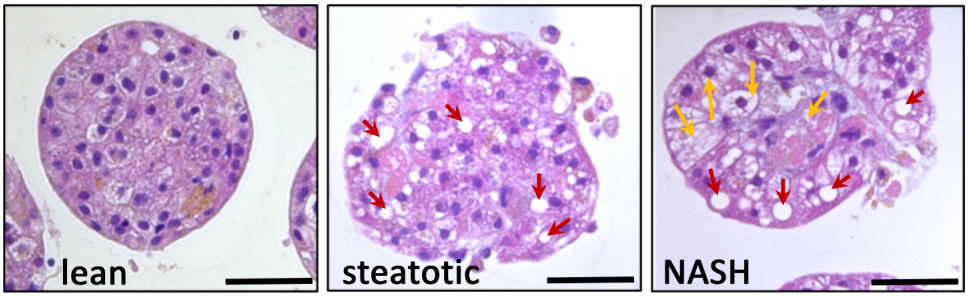
The solution

Human liver microtissues (3DInSight™ Human Liver Microtissues (MT), InSphero) were composed of primary hepatocytes, Kupffer cells, endothelial and stellate cells, representing: 1) healthy, 2) steatotic and 3) pre-fibrotic NASH conditions by applying different media compositions (low and high carbohydrate) without and with lipid supplementation and/or LPS treatment.

The different phenotypes were clearly distinct and revealed histological features and gene expression profile similar to clinical *in vivo* samples from respective disease states.

Human liver microtissues representing healthy, lean state (left), steatotic (middle) and NASH phenotype (right) with typical histopathological features such as lipid droplets (red arrows) and ballooning hepatocytes (orange arrows) Bar = 50 µm



Outcome

Human liver microtissues where exposed to a panel of NMs (ZnO, CeO2 and TiO2) for up to 3 weeks, including recovery periods of 1-2 weeks, applying very low sub-lethal *in vivo* relevant concentrations (1.25-5 µg/ml) with following findings:

- Little NM-induced cell death was detected, and this was mostly masked by the MT ageing effect beyond 2 weeks of treatment.
- Pro/anti-inflammatory protein secretion showed time dependent NM-induced increases in cytokine levels.
- Upon NM treatment, liver tissues showed a progressive effect with regard to cell death and cytokine release, relative to the disease stage (lean < steatotic < NASH).
- While healthy liver showed full recovery, this was not the case in diseased steatotic and NASH liver with sustained cytokine release during the entire wash-out phase.
- NM treatment led to an activation of liver stellate cells (mediators of progressive liver disease) as measured by alpha-SMA protein levels.
- Potential novel biomarker identified for NM-induced adverse liver effects (CX32)

Kermanizadeh A, et al., 2019. Assessment of nanoparticle-induced hepatotoxicity using a 3D human primary multi-cellular microtissue exposed repeatedly over 21 days - suitability of the in vitro test system as an *in vivo* surrogate. Particle and Fibre Toxicology 16: 42

Kermanizadeh A, et al., 2021. Particulate and drug induced toxicity assessed in novel quadruple cell human primary hepatic disease models of steatosis and pre-fibrotic NASH. Manuscript in submission

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