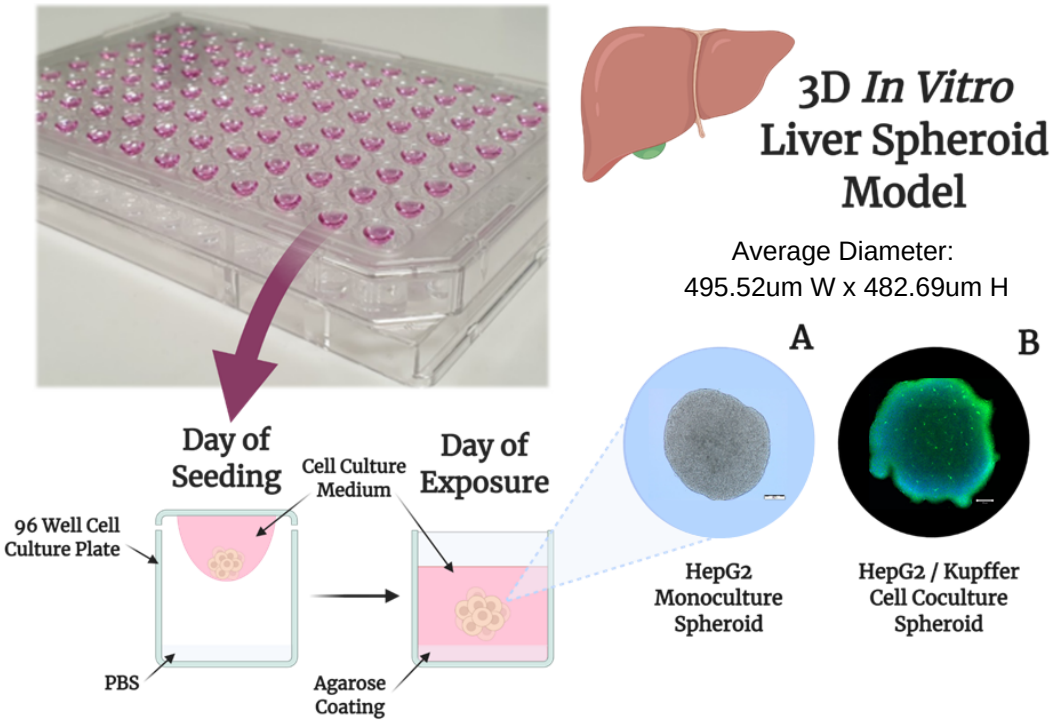
Cell-line based 3D Liver models that can be utilised for nanomaterial toxicity and genotoxicity assessment *in vitro*

Background

Hepatic toxicology is key when considering engineered nanomaterial (ENM) exposure, as it widely known that the liver is a major site of ENM accumulation post exposure. The liver serves a vital role in metabolic homeostasis and detoxification, thus it is imperative that robust and physiologically representative models for ENM liver hazard assessment *in vitro* are established.



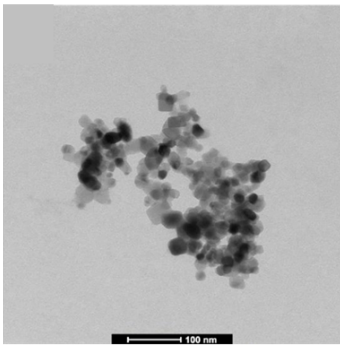


Figure 2: TEM Image of TiO_2 ENMs

Figure 1: Illustration of a 3D *in vitro* liver spheroid model developed by Llewellyn *et al.*, 2020, and formed *via* the hanging drop technique. Microscopy images of (A) HepG2 monoculture and (B) HepG2/Human Kupffer cell coculture spheroid. Green fluorescence represents the CD68 (ab222914, Abcam, UK) positive staining for the human Kupffer cells, whilst the blue fluorescence signifies the DAPI nuclear staining. The scale bars represent 100 µm.

Application

In vitro 3D hepatocyte models (Fig. 1) were developed using an immortalised cell line (HepG2), which are viable for long-term culture (>14 days) and able to support both acute, long-term and repeated ENM exposures. Their ability to predict a range of toxicological endpoints has been characterised using a variety of ENMs (*e.g.* TiO_2 (Fig. 2), ZnO, Ag, $BaSO_4$ & CeO_2) across low-dose exposure regimes.

- Liver function
- (Pro-)inflammatory response
- Cytotoxicity
- Genotoxicity (Fig. 3)

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

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Advanced Tools for NanoSafety Testing

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Results

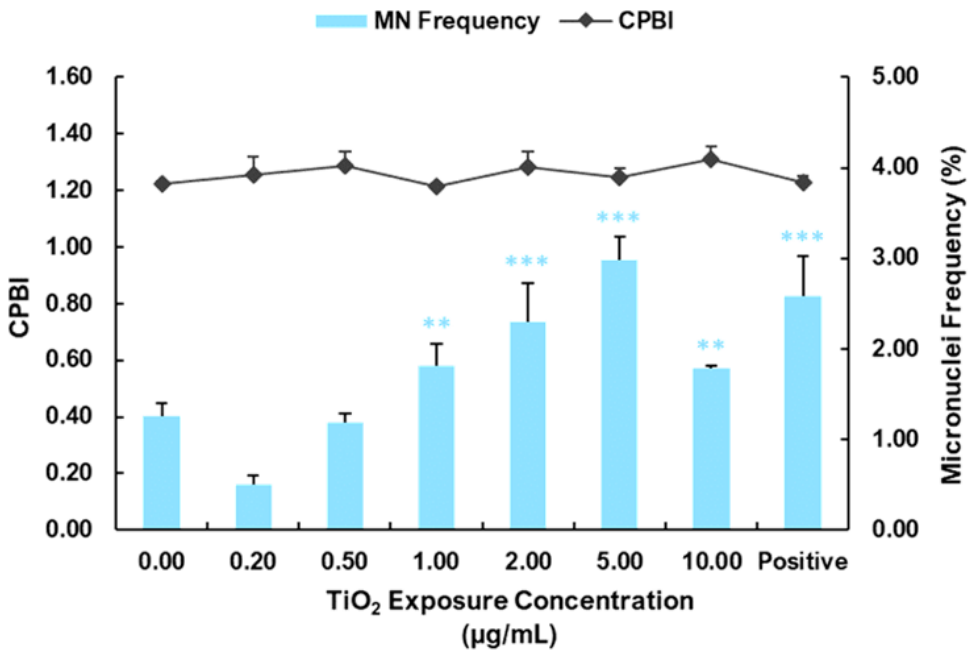

Figure 3: Cytotoxicity (CPBI) and genotoxicity (micronuclei frequency) assessment using the micronucleus assay post acute (24hr) exposure to TiO_2 ENM. Mean data (n=2) presented ± SEM. Significance indicated in relation to the negative control: * = *p≤ 0.05*.

Conclusion

- Advanced 3D *in vitro* liver model able to support a range of toxicological endpoints for ENM Screening, including fixed DNA damage assessment (Fig. 3).
- This 3D liver model has been successfully transferred to multiple independent laboratories.
- An SOP for more physiologically relevant ENM dosing regimes, with a tiered testing strategy to assess ENM biotransformation has been developed.
- This 3D liver model can be adapted to include additional cells types or for high-throughput testing in 384-well formats.

This factsheet is based on the publication Llewellyn, S. V., Conway, G. E., Shah, U. K., Evans, S. J., Jenkins, G. J. S., Clift, M. J. D., Doak, S. H. Advanced 3D Liver Models for In vitro Genotoxicity Testing Following Long-Term Nanomaterial Exposure. J. Vis. Exp. (160), e61141, doi:10.3791/61141 (2020).

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