Assessing genomic instability in 3D hepatocellular spheroids using the *in vitro* alkaline comet assay

Michael J Burgum[1], Elisabeth Elje[2], Melissa Tutty[3], Gillian E Conway[1], Shareen H Doak[1].

[1] *In Vitro* Toxicology Group, Institute of Life Sciences, Faculty of Medicine, Swansea University, Singleton Park, Swansea, SA2 8PP.

[2] The Health Effects Laboratory, The Institute for Air Research, Kjeller, Norway.

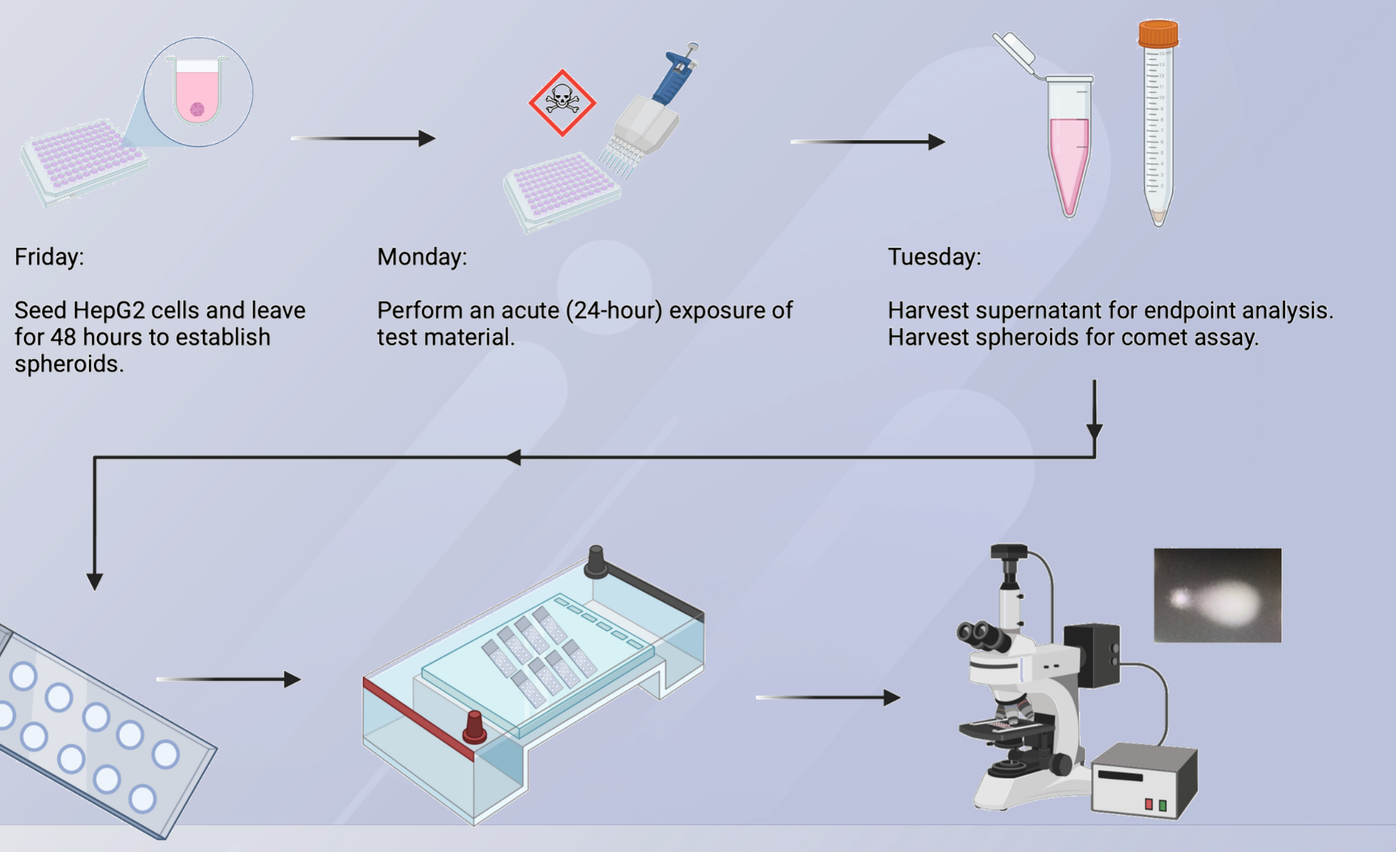
[3] Nanomedicine and Molecular Imaging Group, Institute of Molecular Medicine, Trinity Centre for Health Sciences, James' Street, Dublin 8, Ireland, D08 W9RT.

Background

- Assessing genomic instability using traditional models may not necessarily be as predictive as we need them to be to replace and reduce *in vivo* testing of genotoxic and carcinogenic materials.
- The advantage of the human hepatocellular carcinoma (HepG2) cells are that when cultured as spheroids in a 3D format they can demonstrate liver-like functionality and metabolic activity.
- These crucial features can be used to support genotoxicity testing of chemicals, nanomaterials and pro-carcinogens.
- Moreover, by including the use of the ultra-low attachment plates, these HepG2 spheroids can be grown, exposed, and then analysed in rapid time.
- The aim of this study was to establish a protocol to undertake genotoxicity testing via the *in vitro* alkaline comet assay using 3D HepG2 liver spheroids.

Methods

- Cells were seeded into ultra-low attachment (ULA) plates at 2500 cells/well and cultured for one week.
- The spheroids were treated for 24-hours with 300 μM methyl-, and ethyl-methanesulfonate (MMS and EMS).
- Cytotoxicity was assessed before the spheroids were prepared for the *in vitro* alkaline comet assay. The average %Tail DNA intensity median was calculated using the Comet IV software. A total of 150 cells were scored per biological replicate (*n*=3)



Results

- Following a 24-hour exposure to MMS a statistically significant ($p \leq 0.05$) cytotoxic response was observed (**A**) with a 30% reduction in viable cells compared to the untreated spheroids.
- Statistically significant ($p \leq 0.05$) strand breaks (SBs) were observed using the comet assay in spheroids treated with both MMS and EMS (**B**) inducing 40% and 30% SBs respectively; indicative of a potent genotoxic response and in the case of MMS, correlating to a cytotoxic effect.

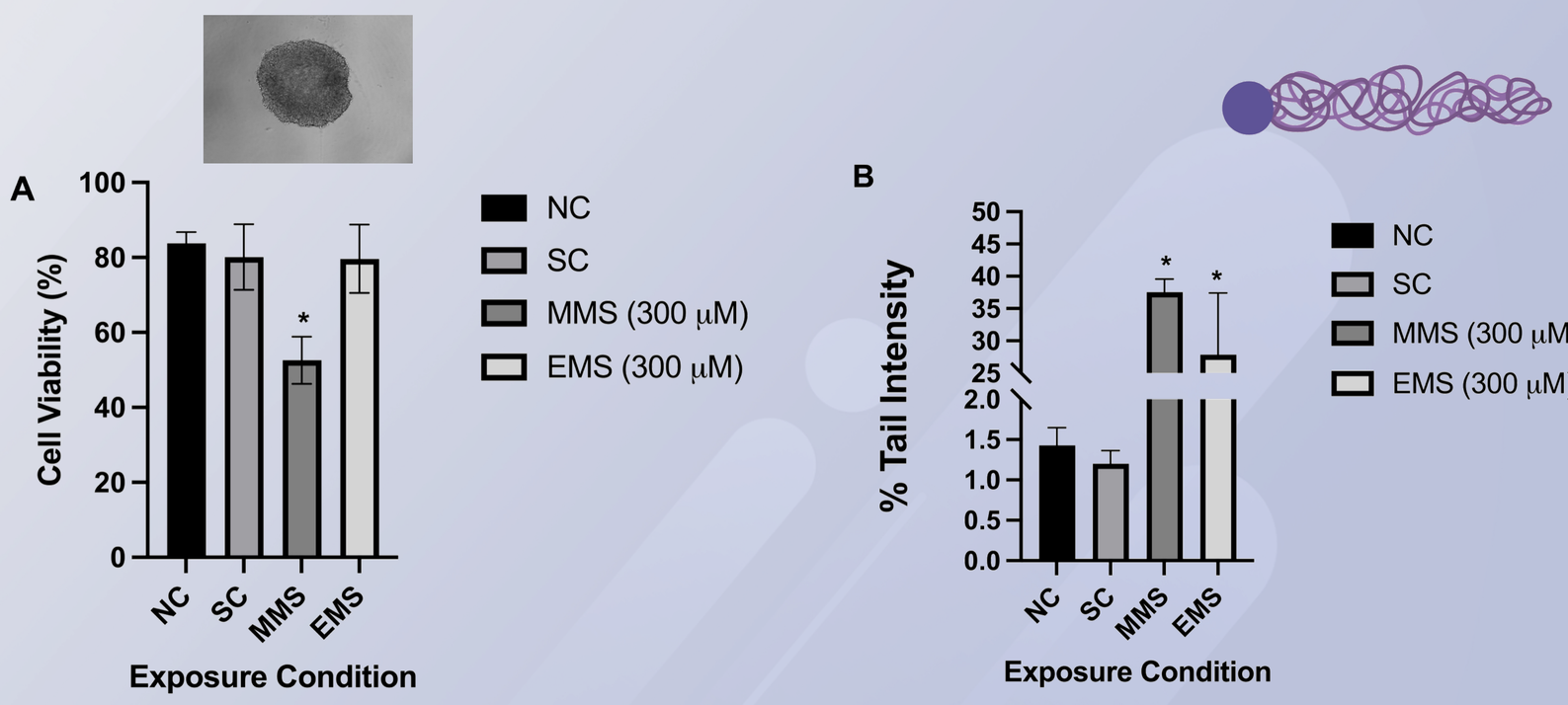


Figure 2. HepG2 spheroid cell viability (A) and % Tail DNA Intensity (B) in the comet assay. The average % Tail DNA Intensity median was calculated for 150 cells per biological replicate, *n*=3.

Outcomes

- Using the ULA plates improved the efficiency of generating HepG2 spheroids for genotoxicity evaluations.
- The *in vitro* alkaline comet assay could be readily applied for genotoxicity testing in this more complex *in vitro* test model.

This research has been funded by the European Commission through the United Kingdom Research and Innovation [Grant Agreement Number 101137613].
I would like to acknowledge and thank UKEMS for awarding the UKEMS conference bursary to attend the 2024 conference 2024.