PATROLS

Advanced Tools for NanoSafety Testing

Liver (commercial & cell line models) and gastrointestinal tract models

Presenter: Rob Vandebriel, National Institute of Public Health & the Environment, the Netherlands

Date: September 12, 2019

Place: OECD, Paris, France

1

Would you consider data generated using these models and endpoints? What would be encouraging? What is reducing uncertainty?

2

Which models should be taken forward/accelerated for eventual use in RA?

3

Are the presented models more generally applicable?

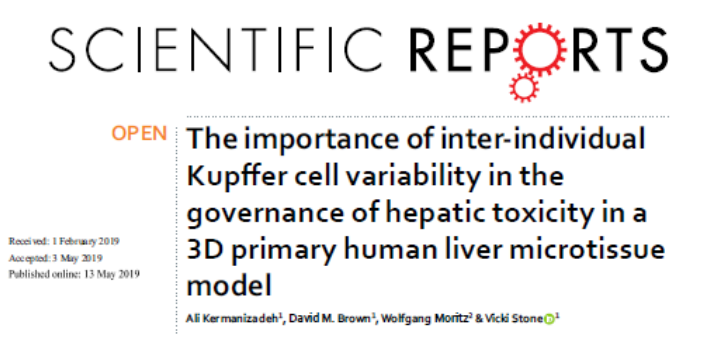
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 760813.

In PATROLS WP4, two liver and two GIT models have been developed. They all allow long-term repeated ENM exposure.

Several are currently being adapted to enhance (patho-) physiological relevance. The aim is to improve hazard endpoint analysis.

1. Human primary liver microtissue (HWU/Insphero)

- Primary human hepatocytes + Kupffer cells + sinusoidal endothelial cells
- Addition of KCs slightly increased toxicity of ENM
- Addition of KCs modified immune responses to ENM
- Inter-individual differences did not prevent immune response conclusions


SCIENTIFIC REPORTS

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The importance of inter-individual Kupffer cell variability in the governance of hepatic toxicity in a 3D primary human liver microtissue model

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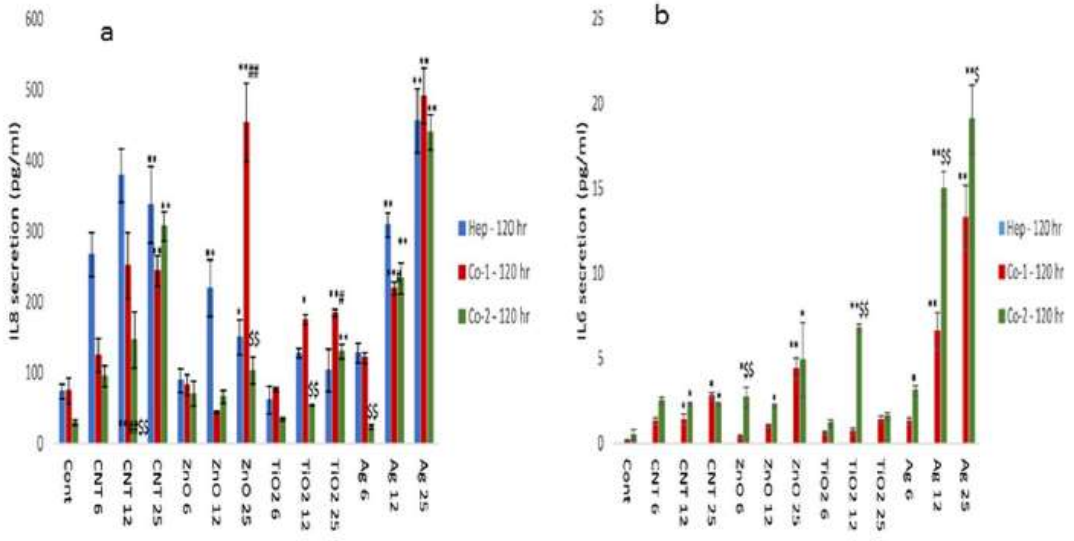

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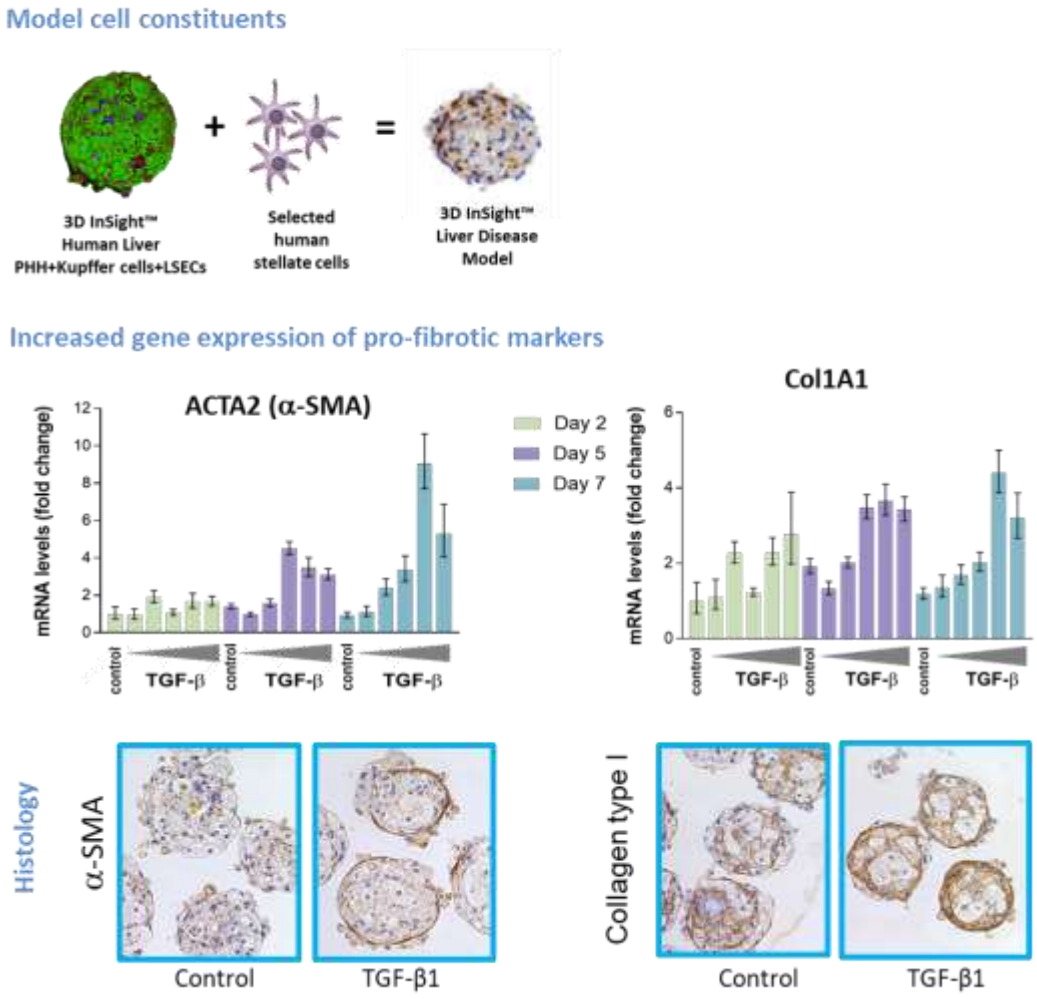
1. Compromised 3D primary human liver microtissue (InSphero/HWU)



- Hepatocytes
- Endothelial cells
- Kupffer cells
- Stellate cells

Range of 'compromised'

- Benign fatty liver to liver
- Liver inflammation
- Liver fibrosis

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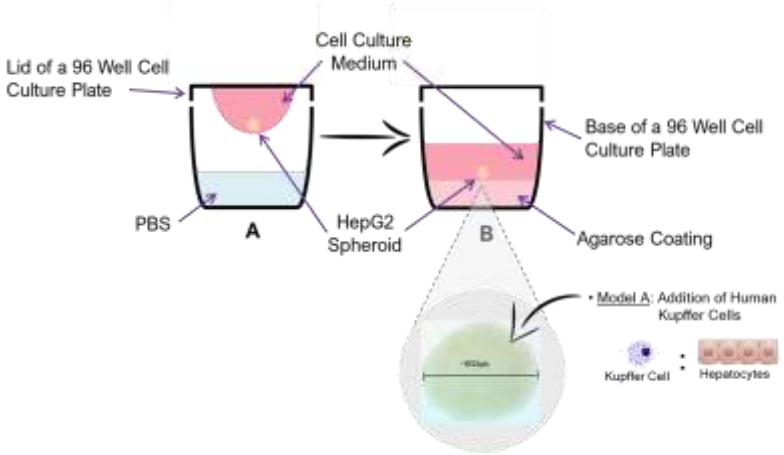
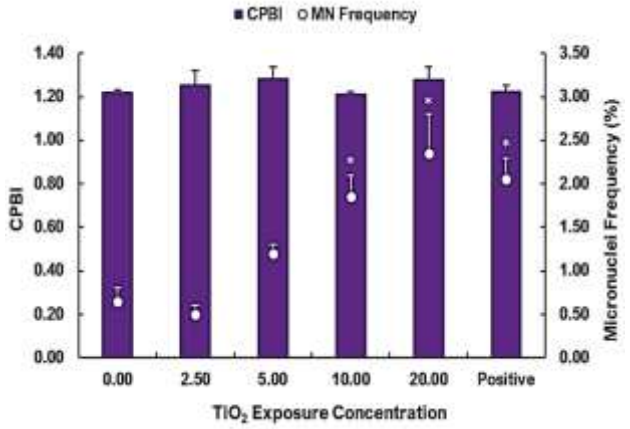
2. Cell line derived liver microtissue (SU)

- Larger than primary cell microtissue in order to generate sufficient cells for genotoxicity
- HepG2 3D spheroids developed (preferred to HepaRG)
- Viable > 14 days
- Retains albumin and urea production
- Retains proliferation
- Exposed to ENM for 1 or 5 days
- Measure liver function, inflammatory responses, cytotoxicity, genotoxicity





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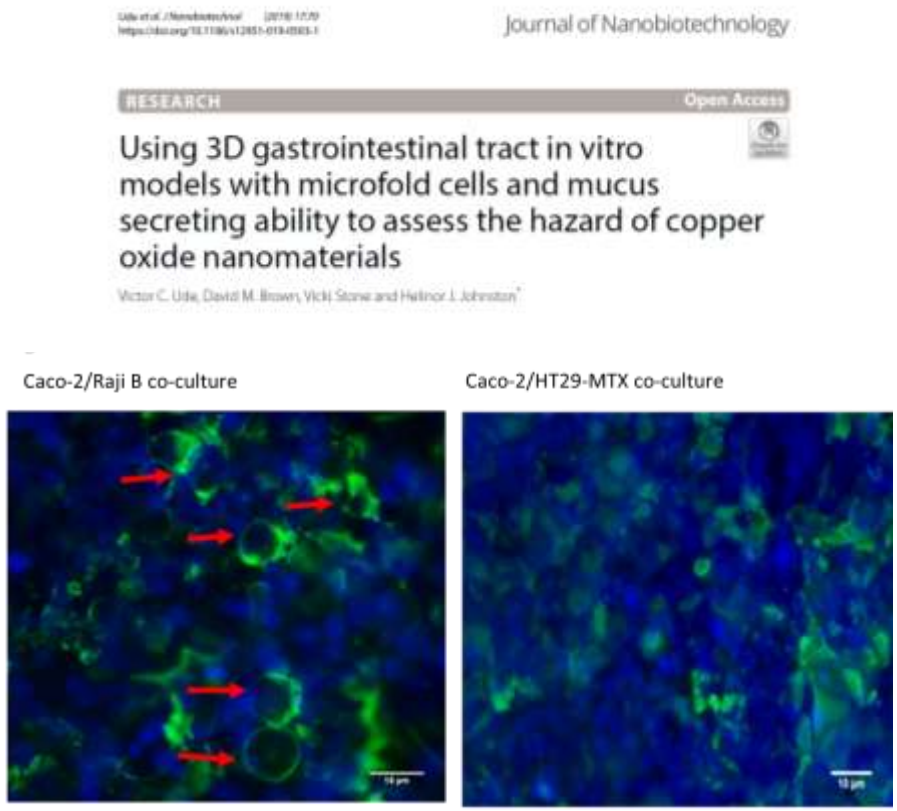
2. Cell line derived liver microtissue (SU)

- Co-culture with primary human KCs: effect of HepG2/KC ratio
- Compare to monoculture microtissues

- Fluidics-based system to introduce flow under development

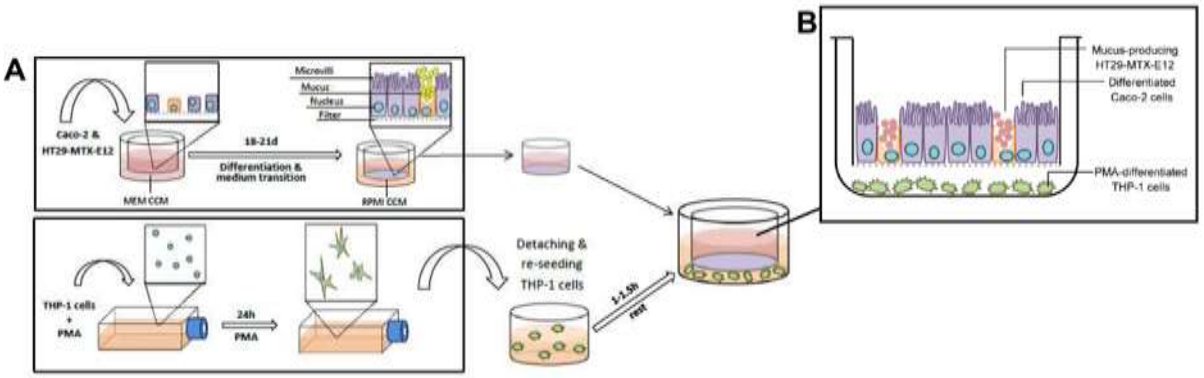
3. GIT Caco2/mucus/M-cell co-culture (HWU)

- Aimed for a 5 day culture, but 21 days required for differentiation prior to the 5 day treatment
  - Caco-2/HT29-MTX (mucus)
  - Caco-2/Raji B (M cell)
  - Caco-2/HT29-MTX/Raji B



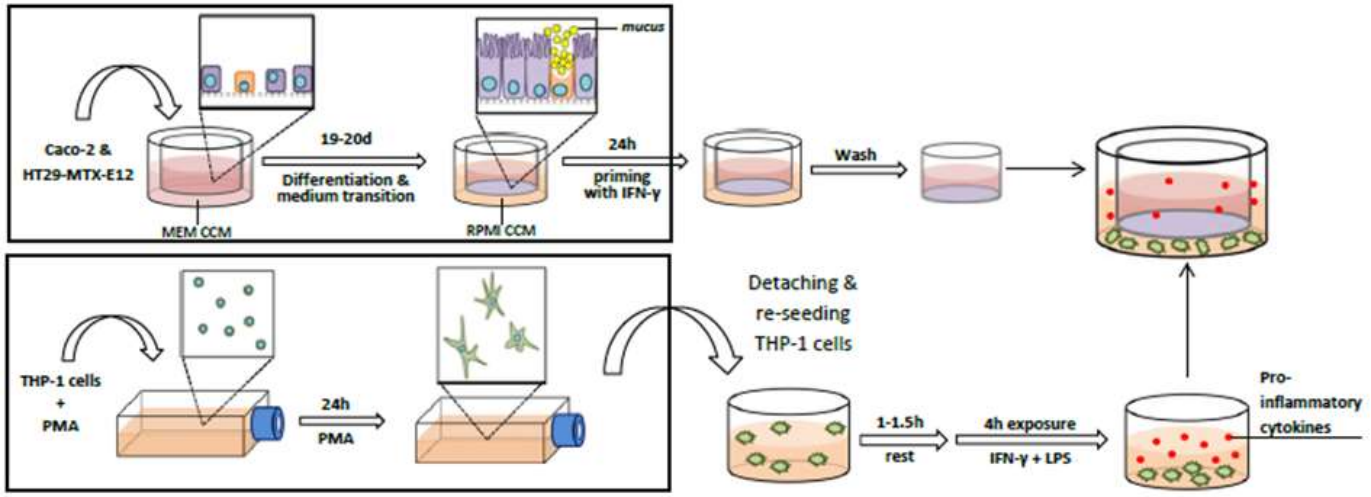
4. GIT macrophage model (IUF)

- Caco-2 + THP-1
- Mucus producing HT29-MTX-E12 added
- Aimed for a 5 day culture, but 21 days required for differentiation prior to the 5 day treatment
- Cytotoxicity, DNA damage, pro-inflammatory potential and gene expression analysis



4. Inflamed GIT (IUF)

- Macrophages will be activated with a cocktail of stressors prior to addition into the co-culture.



Conclusions

- Four protocols that have potential for standardisation (ISO or OECD)

- Liver human primary microtissue model, with repeated exposures for up to 21 days
  - Most advanced protocol for the liver, but cost may be an issue

- GIT Caco2/mucus/M cell co-culture
  - Most advanced GIT model

THANK YOU FOR YOUR ATTENTION

What is your opinion on the models presented? Endpoints?

<u>Towards OECD GD or TG:</u>

- Which one(s) holds most value?
- Do they fill a gap?
- Which one(s) has priority for further development?

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