PATROLS
Advanced Tools for NanoSafety Testing

Bridging the *in vitro–in vivo* divide for hazard testing of nanomaterials

Shareen H. Doak[1] & Barbara Rothen-Rutishauser[2]

1. *Institute of Life Science & Centre for NanoHealth, Swansea University Medical School, Singleton Park, Swansea, SA2 8PP, UK*

2. *Adolphe Merkle Institute, University of Fribourg, Chemin des Verdiers 4, CH-1700 Fribourg, Switzerland*

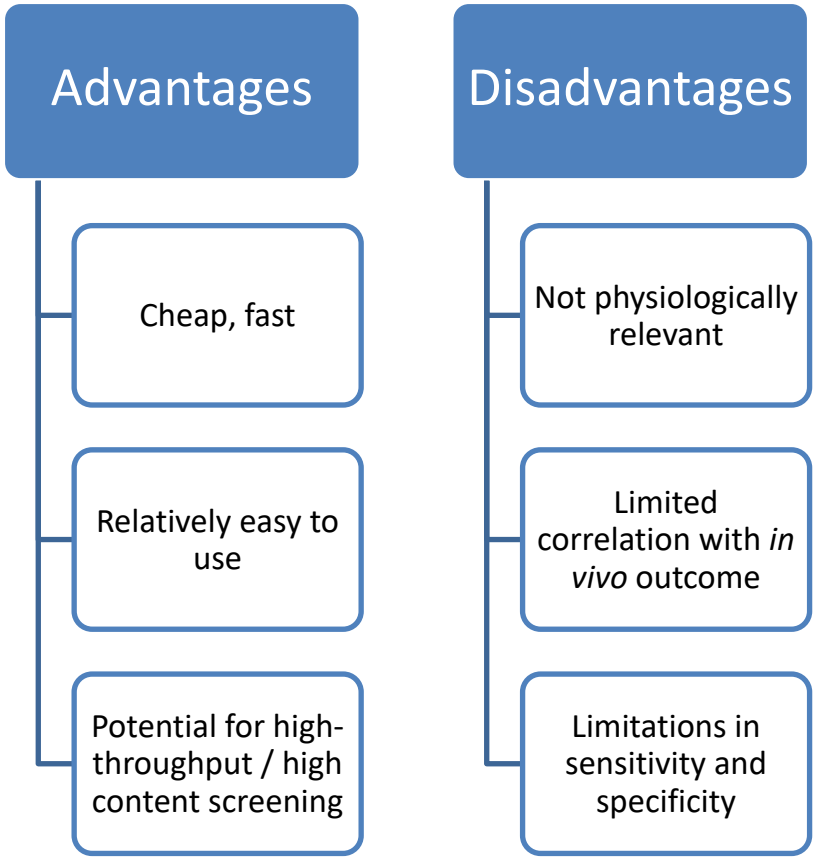
Overview presentation

- 2D versus 3D cell models
- Some examples for:
  - Lung models
  - Skin models
- Introduction to the PATROLS project

Intro to benefits of 3D models / need for new approaches

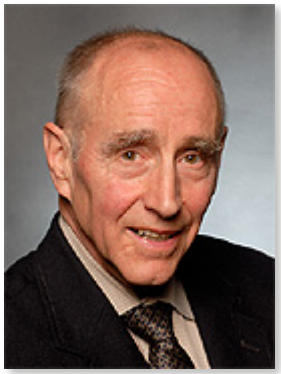
- Monolayer growth of cells (2D) is far removed from *in vivo* state.
- 3D & co-culture testing systems are more physiologically relevant:
  - Show improved metabolic capacity
  - More natural cell-cell / cell-matrix interactions
  - Demonstrate closer *in vivo* behaviours (gene expression; protein function; differentiation; morphology).
  - Varying degrees of complexity from single cell type spheroids to complex multi-cellular structures.
- (Geno)toxicology: potentially reduce mis-leading positives.

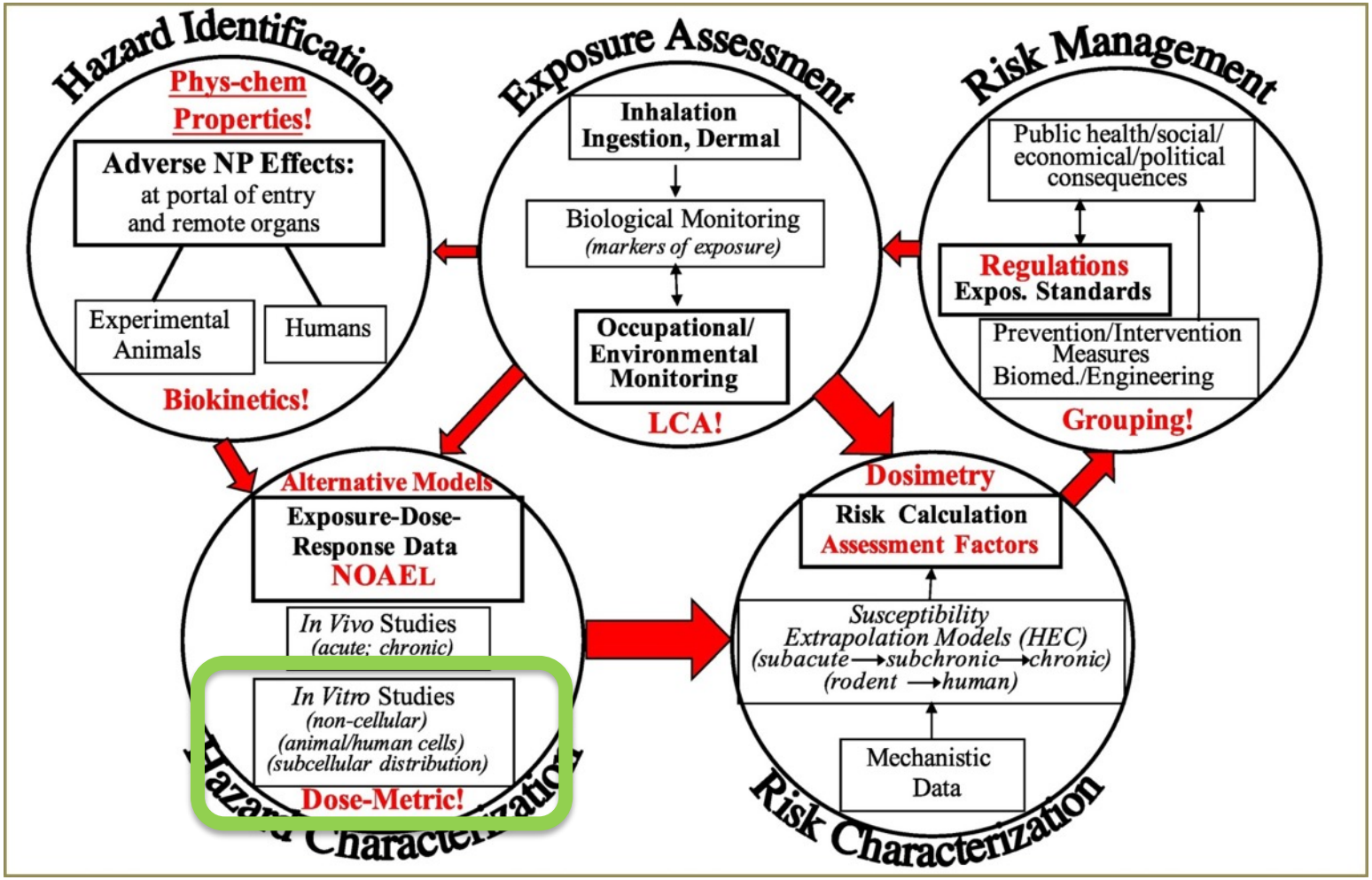
New generation of *in vitro* test systems are required



1. *In vivo* tests are expensive & time consuming
2. EU Cosmetics Directive prohibits animal use for genotoxicity testing since March 2009
3. Shared 3Rs vision

Predictivity of *in vitro* test systems





Oberdörster and Kuhlbusch, NanoImpact 2018

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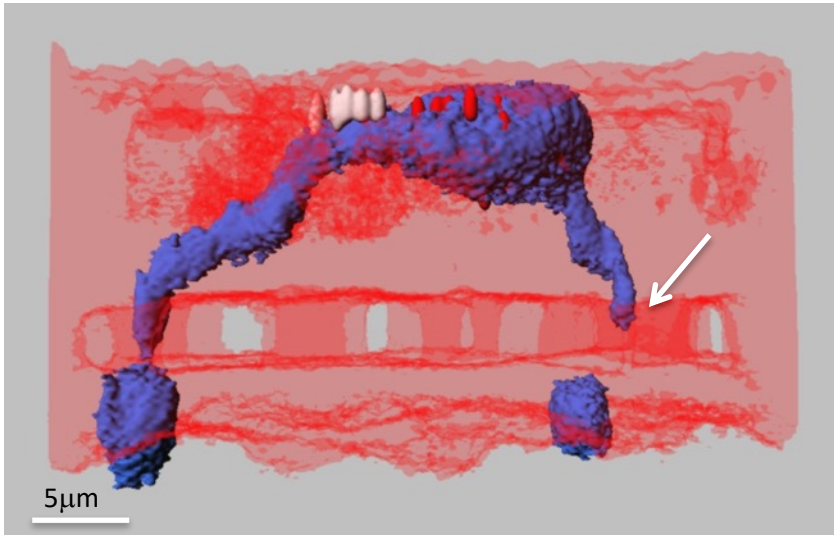
3D Lung cell models

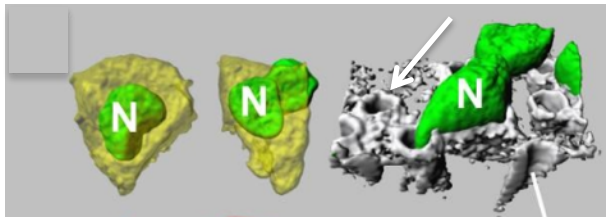
Cell growth on permeable inserts

- Cellular interplay
- Epithelial cell polarisation
- Cell migration assay
- Air-interface

3D Lung cell models

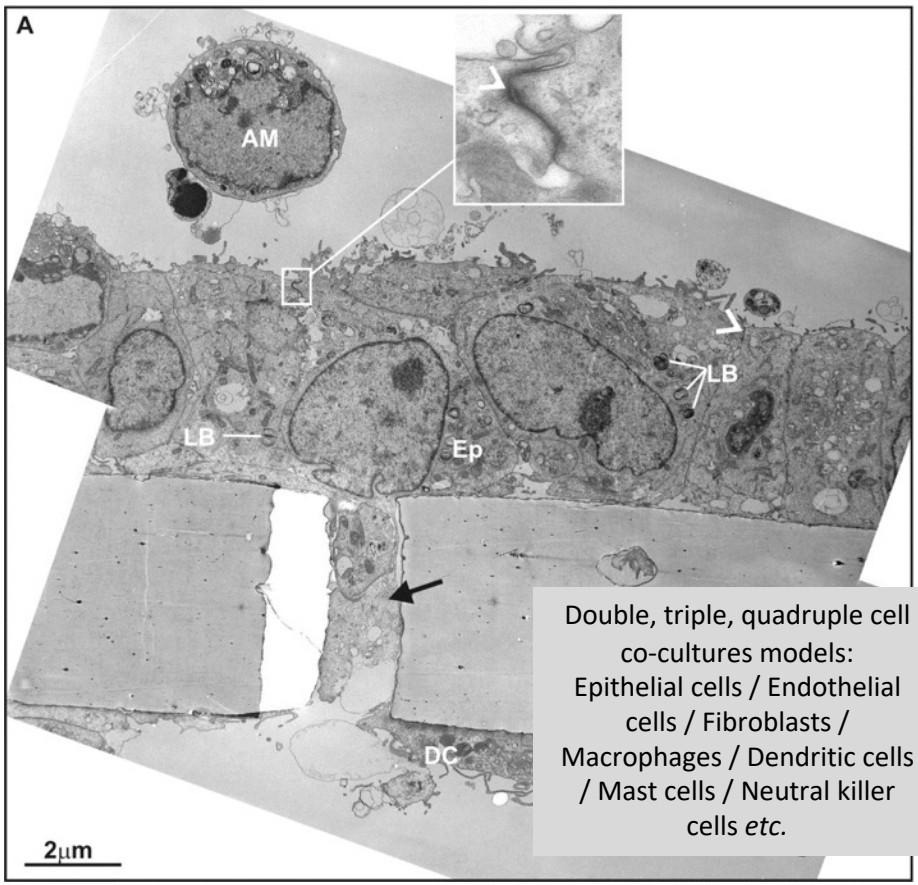
Epithelial cells Macrophages





Nuclei

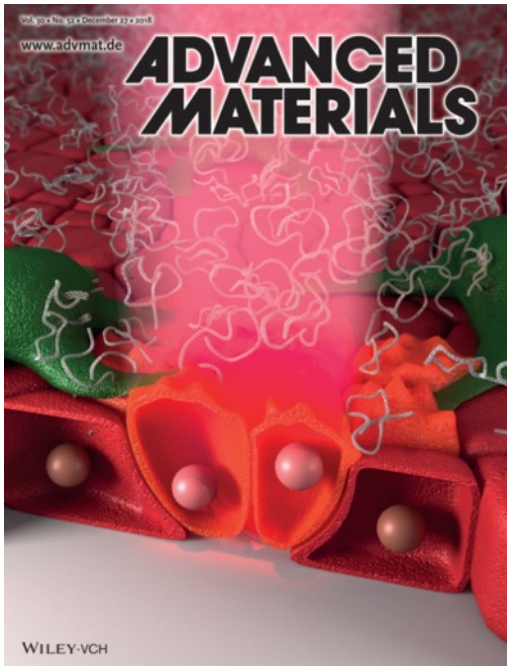
Blank et al. Am J Respir Cell Mol Biol. 2007



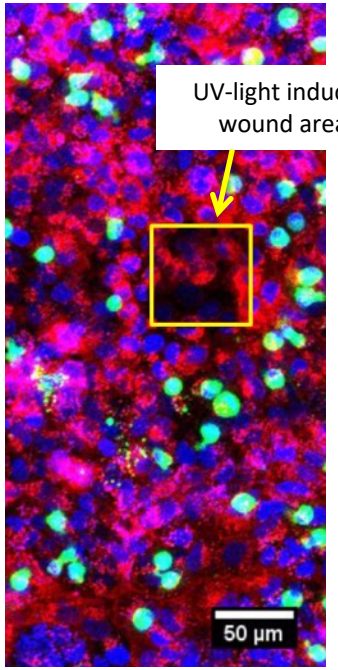
Double, triple, quadruple cell co-cultures models: Epithelial cells / Endothelial cells / Fibroblasts / Macrophages / Dendritic cells / Mast cells / Neutral killer cells *etc.*

Rothen-Rutishauser et al. Am J Respir Cell Mol Biol 2005; Blank et al. Am J Respir Cell Mol Biol 2007; Rothen-Rutishauser et al. Exp Opin Drug Metab Toxicol 2008; Fytianos et al. Nanomedicine (Lond) 2016

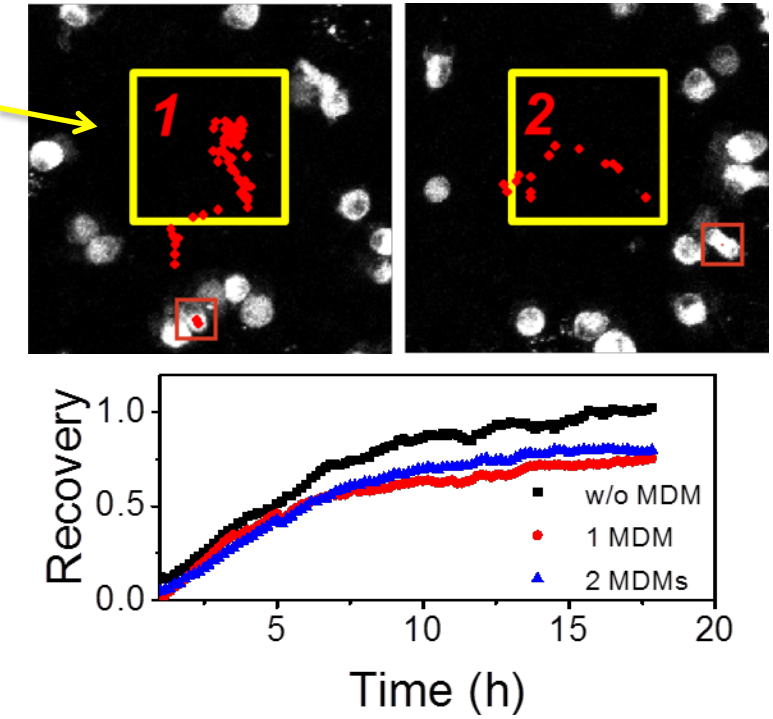
Revealing the Role of Epithelial Mechanics and Macrophage Clearance during Pulmonary Epithelial Injury Recovery



D. Septiadi et al., Adv. Mater. (2018)



A549 lung epithelial cells
Monocyte-derived macrophages (MDM)



Recovery rate of the wound is a function of number MDMs involved in the clearance, however, the reported value was still less than the recovery value of the control sample when no MDMs were involved. We attribute this to a possible competitive effect that exists between live epithelial and macrophages, as both cells need to occupy the injury site in order to clear the dead cells.

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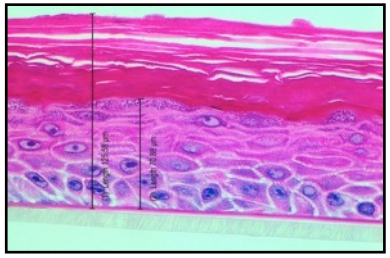
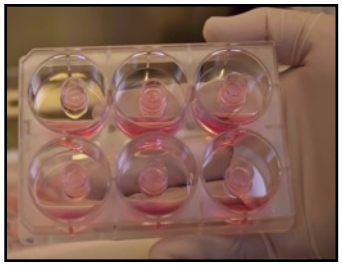
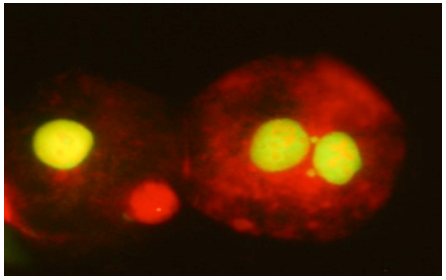
Human 3D Reconstructed Skin Micronucleus (RSMN) Assay

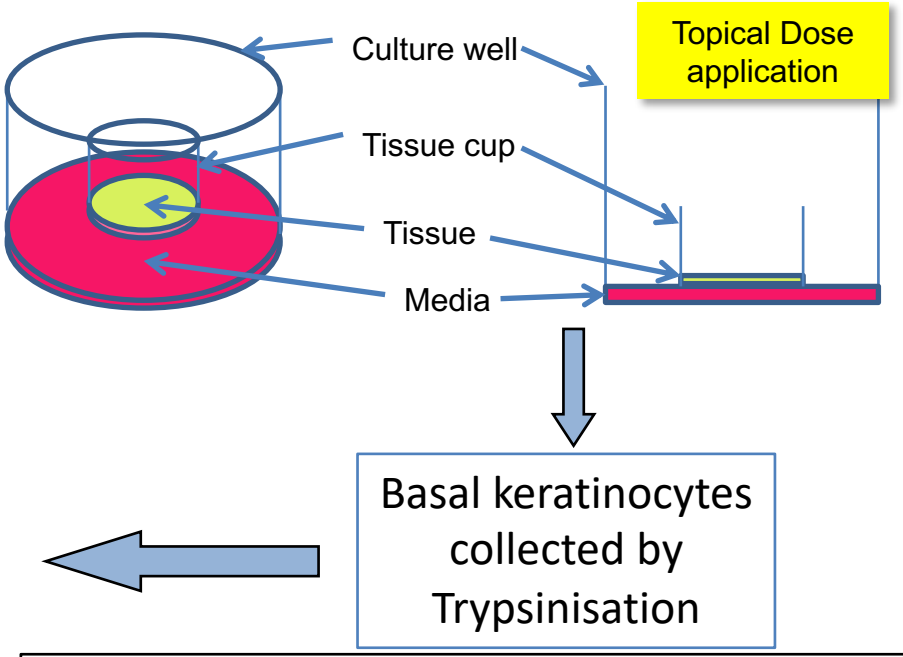
- Promising new *in vitro* genotoxicity testing approach (dermal application).

- 3D RSMN assay suitable for assessing mutagenic hazard: Pfhuler *et al*. Toxicol *in vitro* 28, 18-23, 2014.









Mutagenesis vol. 29 no. 3 pp. 165–175
Advance Access publication 27 March 2014
doi:10.1093/mutage/geu011

Automation and validation of micronucleus detection in the 3D EpiDerm™ human reconstructed skin assay and correlation with 2D dose responses

K. E. Chapman[1,†], A. D. Thomas[1,†], J. W. Wills[1], S. Pfuhler[2], S. H. Doak[1] and G. J. S. Jenkins[1,*]

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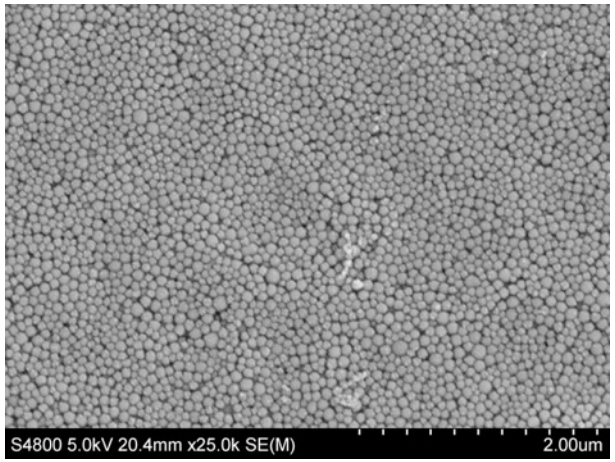
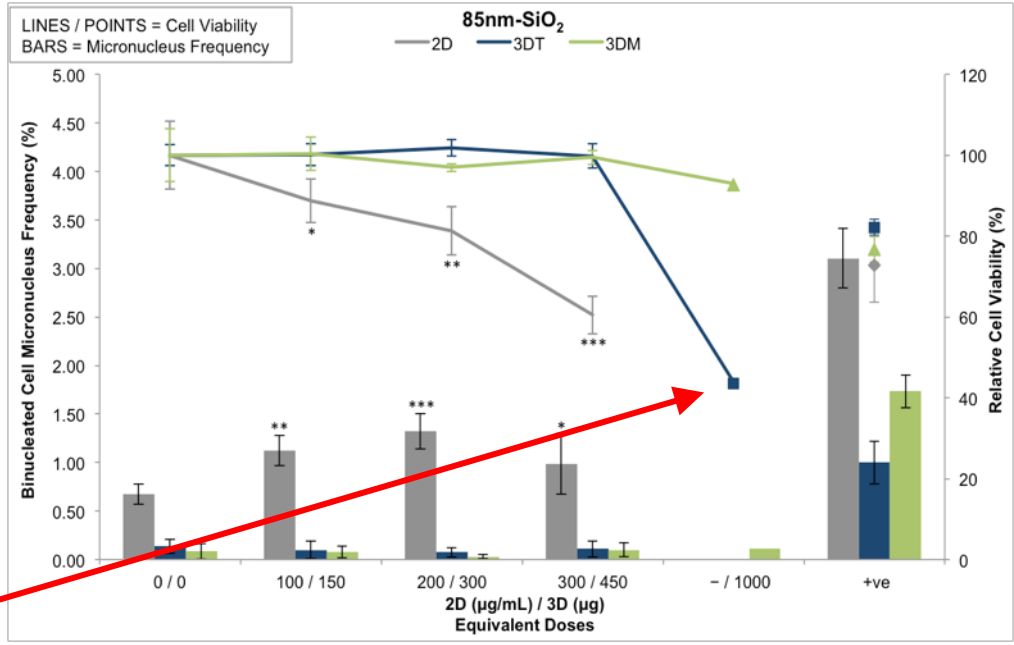
2D vs 3D micronucleus assay

85nm silica

- 2D: cytotoxic & genotoxic
- 3D: no significant cytotoxicity or genotoxicity – model suffocation top dose





Wills et al. Particle and Fibre Toxicology (2016) 13:50
DOI 10.1186/s12989-016-0161-5

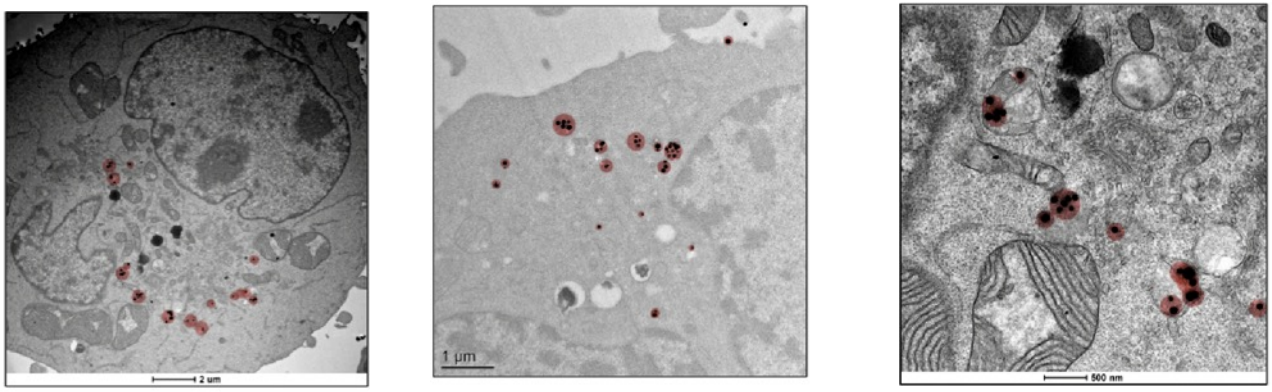
Particle and Fibre Toxicology

RESEARCH Open Access

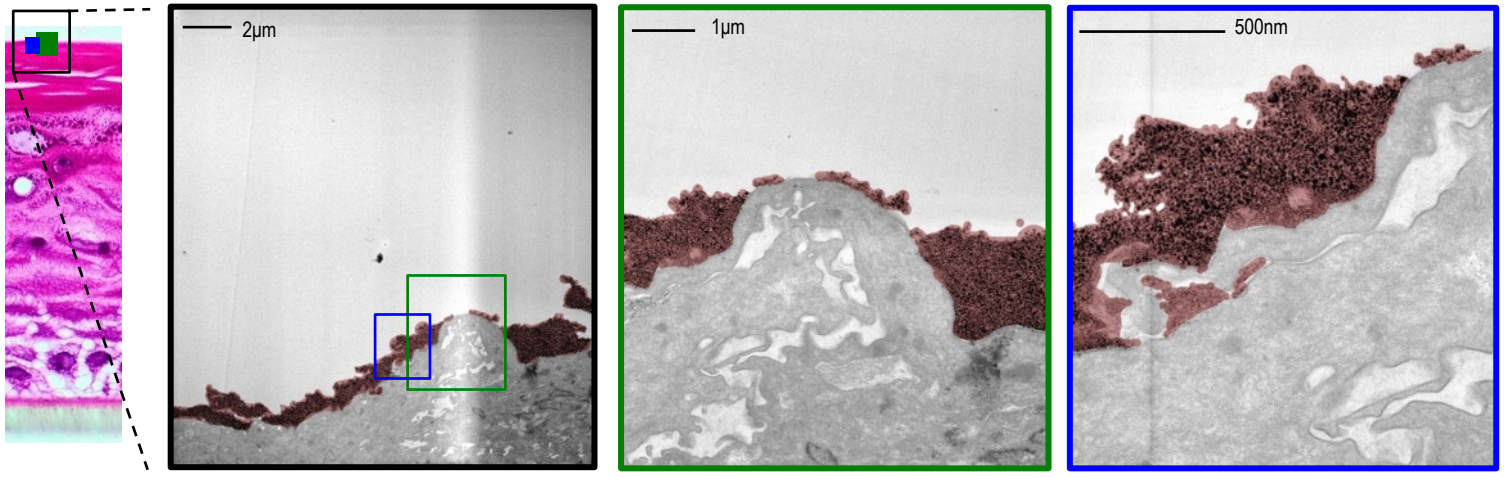
Genetic toxicity assessment of engineered nanoparticles using a 3D in vitro skin model (EpiDerm™)

John W. Wills[1*], Nicole Hondow[2], Adam D. Thomas[1], Katherine E. Chapman[1], David Fish[1], Thierry G. Maffeis[3], Mark W. Penny[3], Richard A. Brown[3], Gareth J. S. Jenkins[1], Andy P. Brown[2], Paul A. White[4] and Shareen H. Doak[1*]

Cell Uptake (16nm Silica)



TK6 cells



3D Epiderm model

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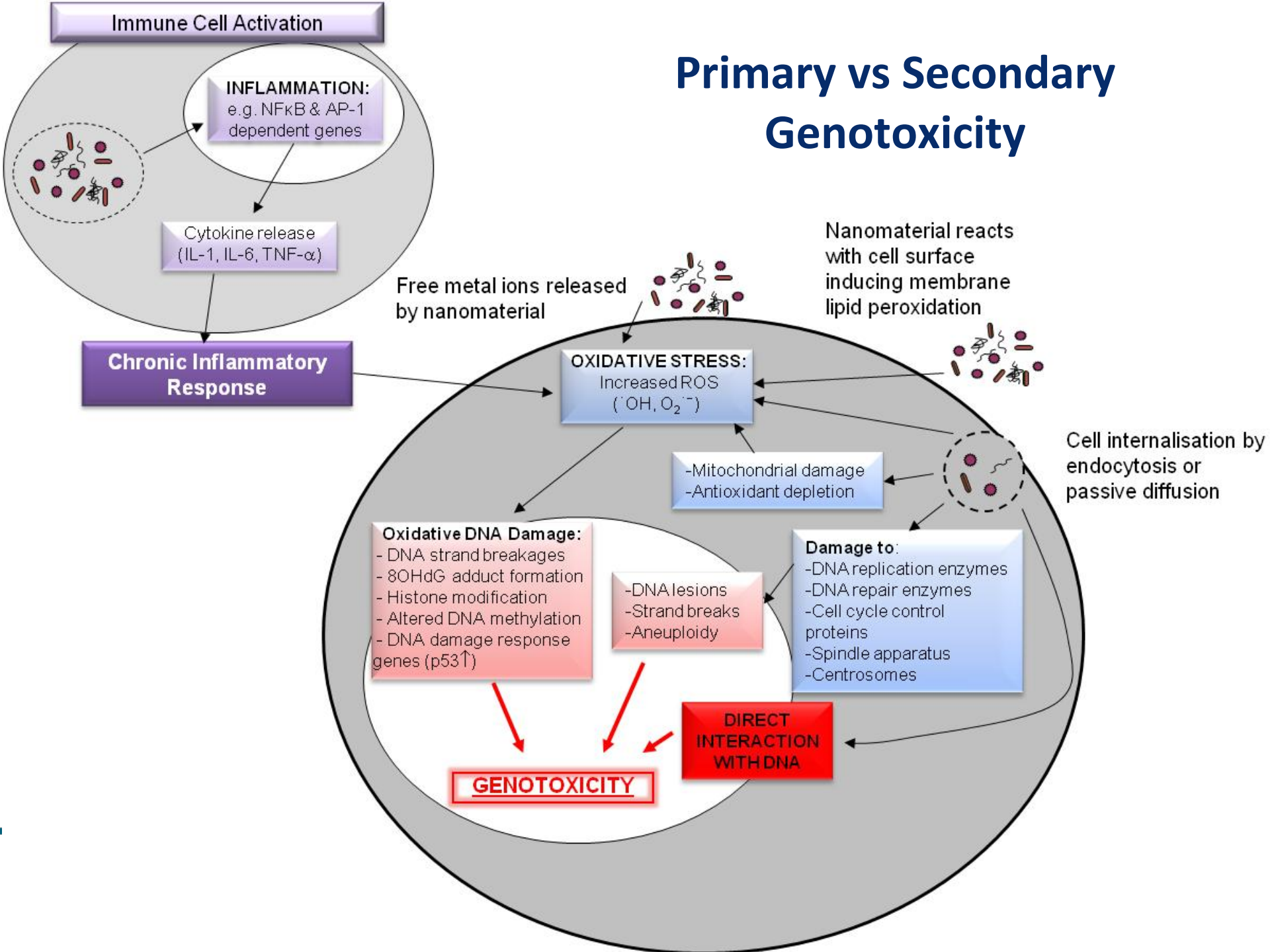
Primary vs Secondary Genotoxicity

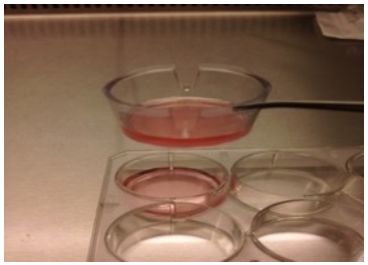


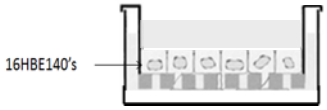
Lung co-culture model: 16HBE14o- epithelial cells with differentiated THP-1 macrophages

A

PET Transwell membrane with 3 µm pores.

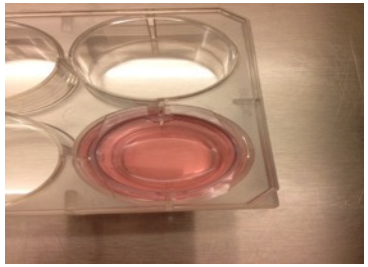
B

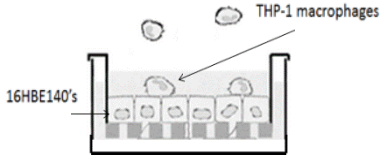




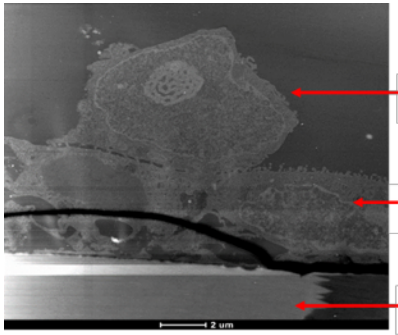
Seed 16HBE14o- cells in upper chamber.

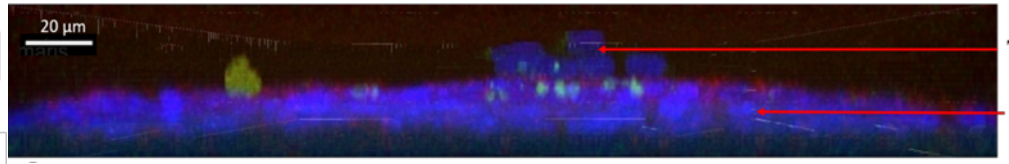
C





Differentiated THP-1 macrophages seeded on top of 16HBE14o-.





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Evans et al. Particle and Fibre Toxicology (2019) 16:8
https://doi.org/10.1186/s12989-019-0291-7

Particle and Fibre Toxicology

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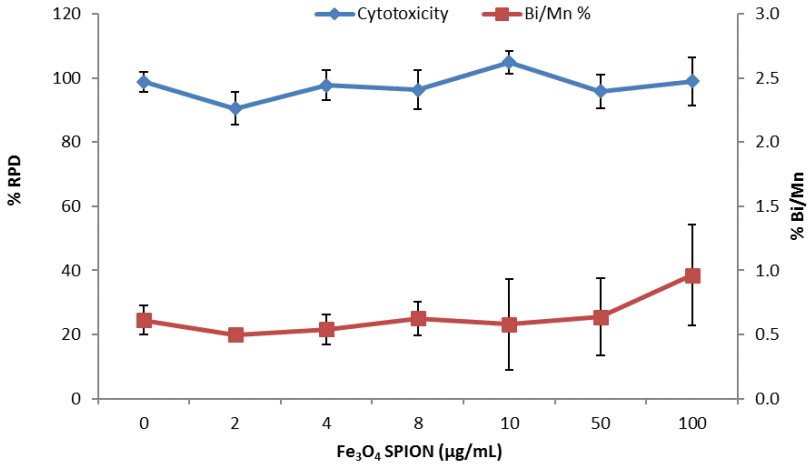
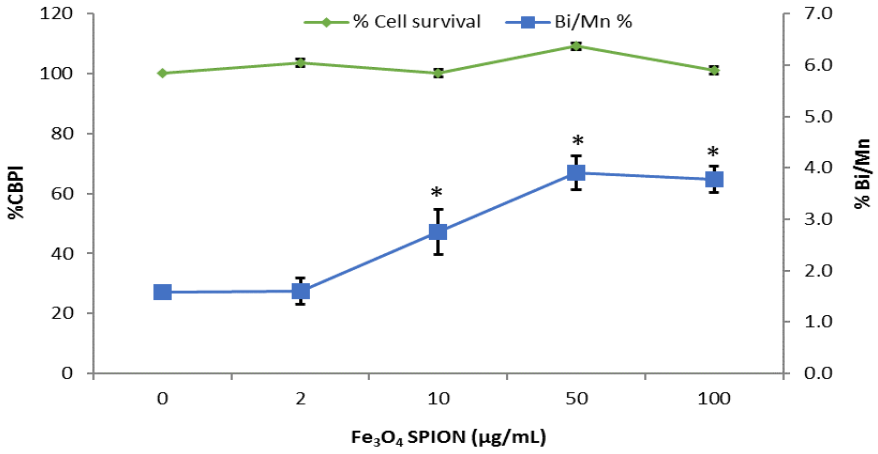
In vitro detection of in vitro secondary mechanisms of genotoxicity induced by engineered nanomaterials

Stephen J. Evans[1], Martin J. D. Clift[1], Neenu Singh[2], John W. Wills[3], Nicole Hondow[4], Thomas S. Wilkinson[1], Michael J. Burgum[1], Andy P. Brown[4], Gareth J. Jenkins[1] and Shareen H. Doak[1*]

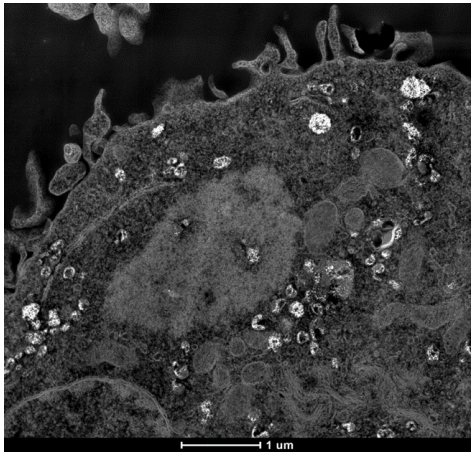
USPION genotoxicity: monoculture CBMN assay vs co-culture Mn assay



Monoculture Mn assay: Fe_3O_4



Co-culture Mn assay: Fe_3O_4



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PATROLS: Physiologically Anchored Tools for Realistic nanOmaterial hazard aSsessment

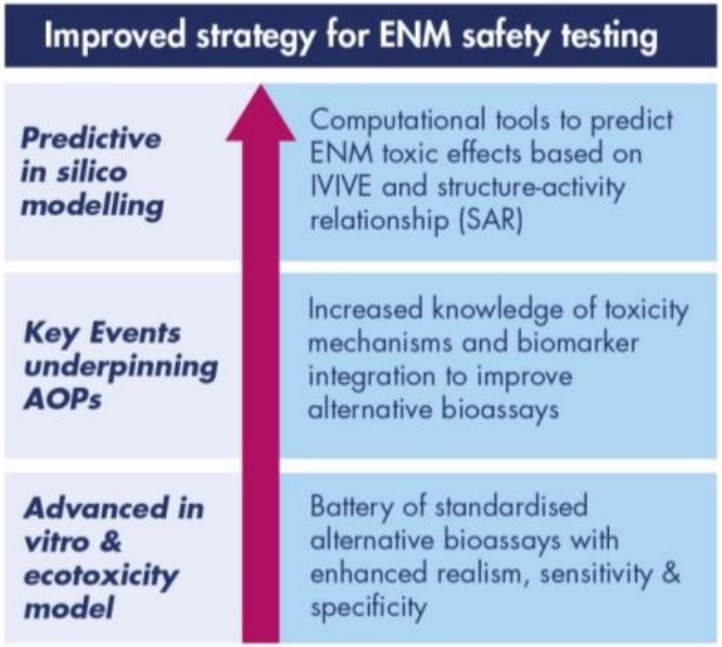
24 partners

14 countries

12.7 million Euros

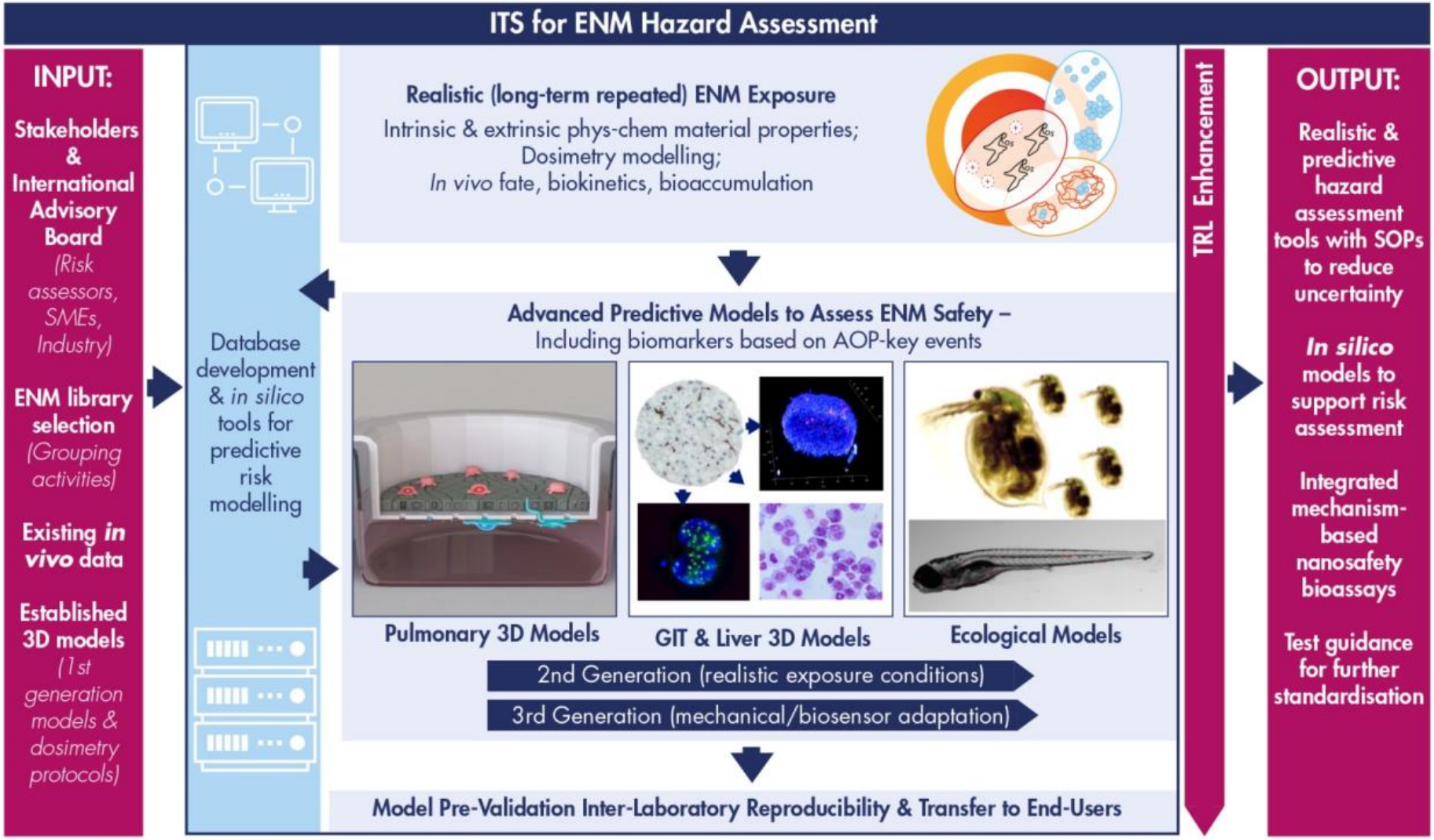
PATROLS aim & vision

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 in human and environmental systems to support regulatory risk decision making.



1st Jan 2018 – 30th June 2021 (42months)

PATROLS Concept



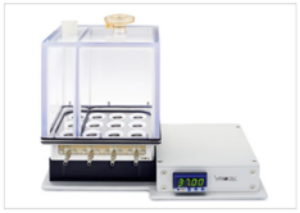
Development of advanced lung models

Evaluating 3 lung cell lines: ability to survive long-term at the ALI & display close to *in vivo* characteristics.

Establishing epithelial & macrophage cell co-cultures.

First ENM aerosolization experiments

VITROCELL® Cloud System



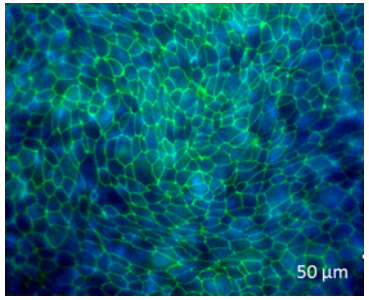
Turnkey exposure system for liquid aerosols with single droplet sedimentation: version for 6, 12 and 24-well sized inserts.

VITROCELL® Powder Chamber

For exposure to smallest quantities of dry powders.

Automated Exposure Station

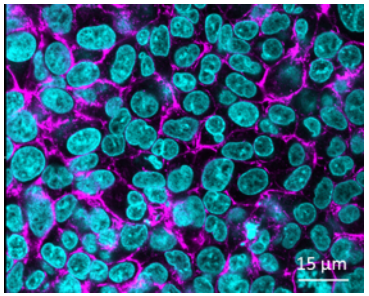
Turnkey Automated Exposure Station with advanced controls.



Calu-3

ZO-1 Nuclei

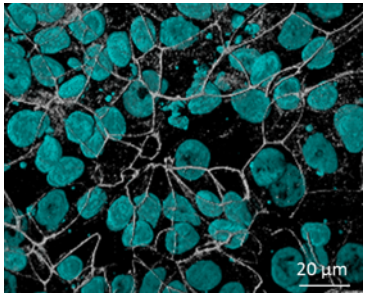
>3 weeks at ALI



A549

F-Actin Nuclei

3 days at ALI



hAELVi

ZO-1 Nuclei

>3 weeks at ALI

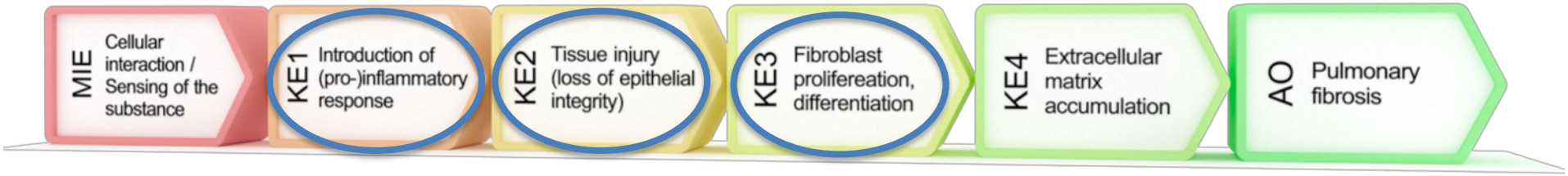
https://www.vitrocell.com/

Development of advanced lung models

Confidential data removed

Development of advanced lung models

- Inflammatory endpoints
  - Viability, proliferation, membrane integrity
  - ROS production, profibrotic mediator release (IL-1β, TNF-α, IL-8, IL-6 and MCP-1)
- Fibrotic endpoints
  - Fibroblast proliferation, α-sma upregulation, collagen production
  - ROS production, profibrotic mediator release (IL-1β, TNF-α, IL-8, IL-6 and MCP-1)

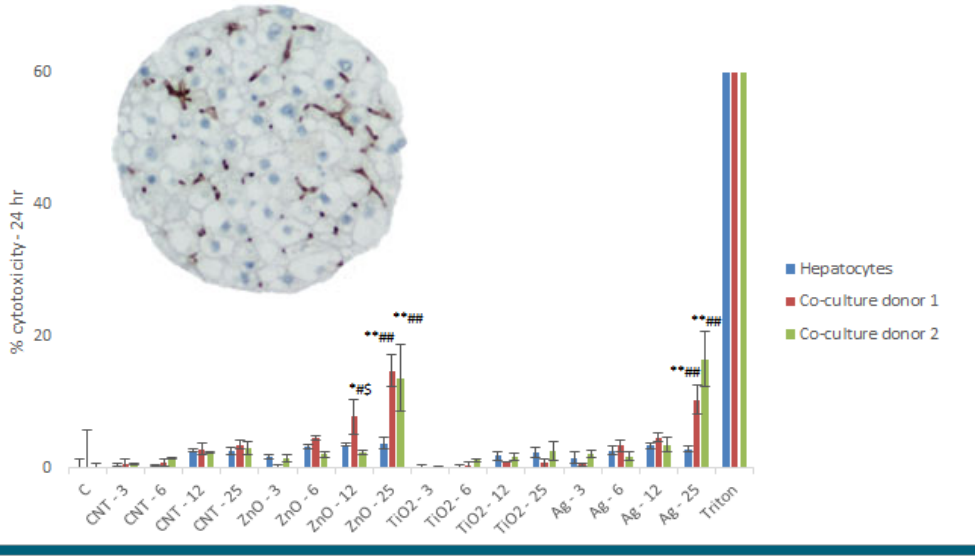


EpiAlveolarTM lung model to predict fibrosis

Confidential data removed

Development of advanced 3D liver models - InSphero primary human microtissues

- Single 24h vs 120h daily repeated exposures (cytotoxicity):
  - Responses to ZnO and Ag greater than for TiO2 and MWNT
  - Responses from coculture are greater than monoculture & modified immune responses to ENM
  - Variable response between donor sources



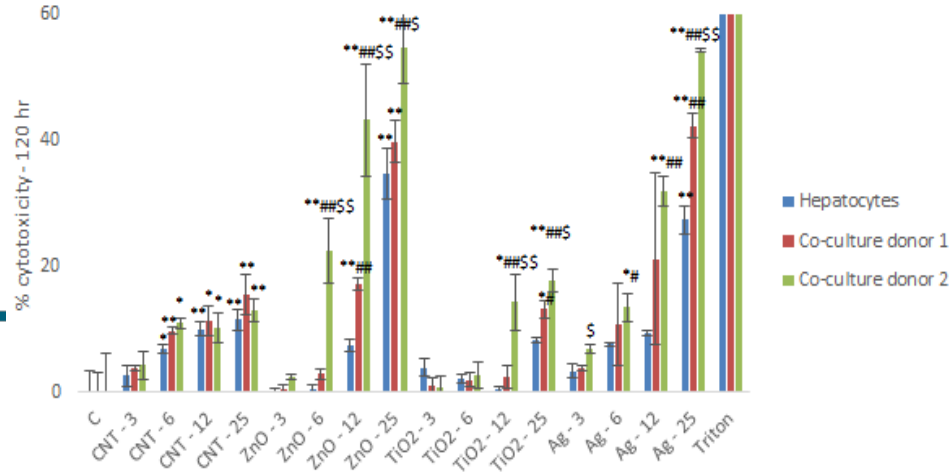
SCIENTIFIC REPORTS

OPEN

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

Received: 1 February 2019
Accepted: 3 May 2019
Published online: 13 May 2019

Ali Kermanizadeh[1], David M. Brown[1], Wolfgang Moritz[2] & Vicki Stone[1]



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Development of advanced 3D liver models – cell line based spheroids

Confidential data removed

Development of advanced gastro-intestinal tract (GIT) models

Toxicology in Vitro 45 (2017) 31–43

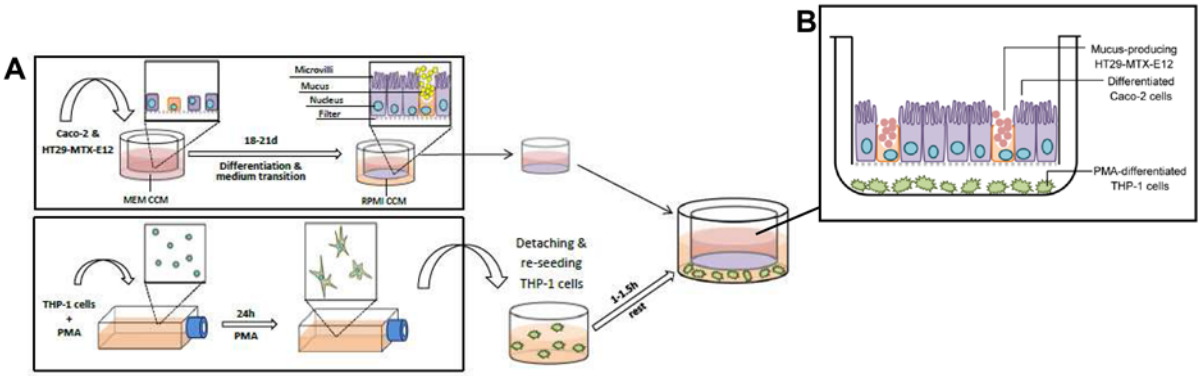
Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Development of an *in vitro* co-culture model to mimic the human intestine in healthy and diseased state

- Caco-2 (enterocytes) + THP-1 (macrophages)
- Mucus producing HT29-MTX-E12 goblet cells added to establish an intestinal triple culture
- 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



Conclusions

- Advanced *in vitro* assays are a promising and relevant new non-animal approach for hazard assessment.
  - Provide more realistic biological barriers
  - Co-culture of multiple cells allows detection of broader range of cell damage mechanisms

- Challenges in 3D model approach:
  - Assay development so models detect wide range of hazard endpoints.
  - Harmonised SOPs & validation is required
  - To accelerate the use of advanced *in vitro* methods open dialogue between relevant stakeholders (academics, regulators, legislators, industrial scientists) is required

Thank you for your attention & questions!

- Website: www.patrols-h2020.eu
- Newsletter: Sign up to follow updates
- LinkedIn Group: www.linkedin.com/company/patrols
- Twitter: @PATROLS_H2020
- PATROLS Project Office: management@patrols-h2020.eu

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