



PATROLS

Advanced Tools for NanoSafety Testing



Swansea
University

Prifysgol
Abertawe

Advanced *in vitro* human tissue models

Lung (WP3)

Kirsty Meldrum

Advanced *in vitro* pulmonary models for engineered nanomaterial hazard assessment

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adolphe merkle institute
excellence in pure and applied nanoscience



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els



misvik biology



Optimise lung models (monocultures)

Optimisation of epithelial lung cell growth in mono-cultures

Bronchial cells

Alveolar cells

Calu-3
ZO-1 Nuclei
>3 weeks at ALI
50 µm

A549
F-Actin Nuclei
3 days at ALI
15 µm

hAELVi
ZO-1 Nuclei
>3 weeks at ALI
20 µm

Trachea, Bronchi, Nonrespiratory bronchioles, Respiratory bronchioles, Alveolar ducts, Alveolar sacs, Lymph nodes, Pulmonary arteries, Pulmonary veins, Alveolar capillary bed

Optimise lung models (co-cultures)

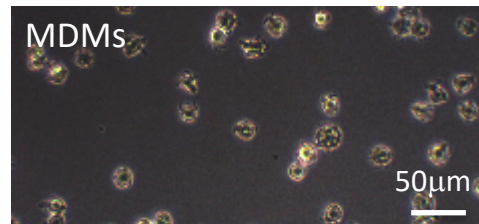
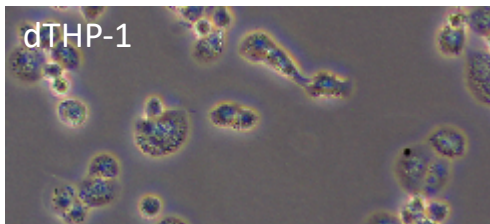
Co-cultures of epithelial cells with macrophages

Number of cells in human lung

Epithelial cells: 38 500 cells/cm²

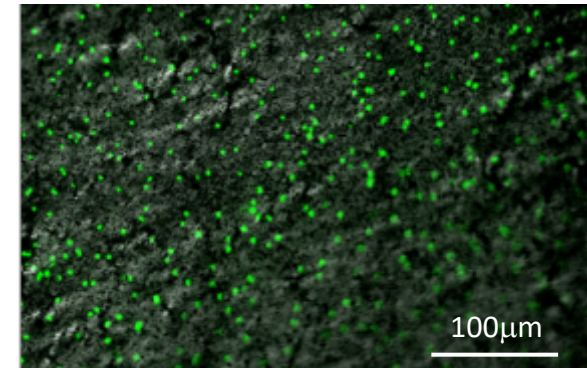
Macrophages: 15 000 cells/cm²

Pinkerton et al., 2015, eBook ISBN: 9780124047266



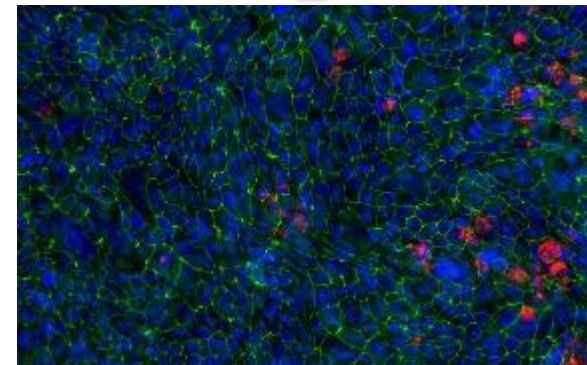
A549 cells

■ MDMs



Calu-3 cells

■ dTHP-1

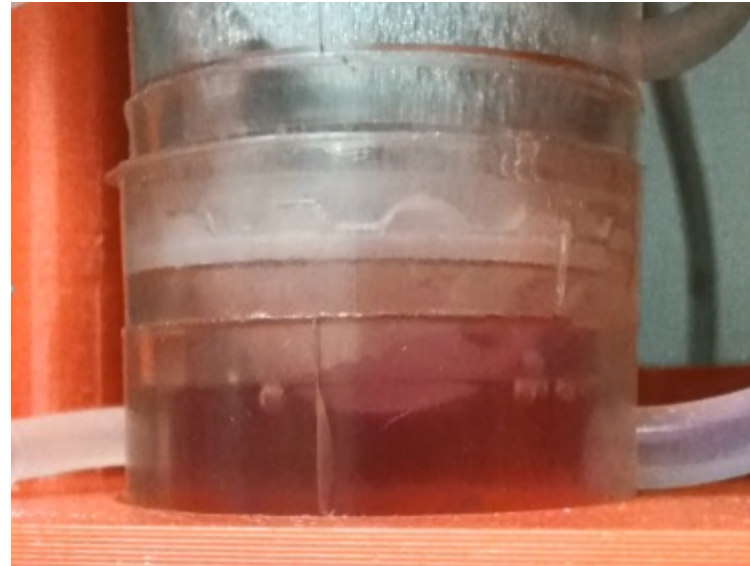


Partner	Co-cultures
AMI	hAELVI/monocyte-derived macrophages (MDMs)
SU	A549/differentiated THP-1 cells (dTHP-1)
RIVM	Calu-3/MDMs or dTHP-1

Strategy for prolonged cultures – repeated addition of macrophages to epithelial tissue each week

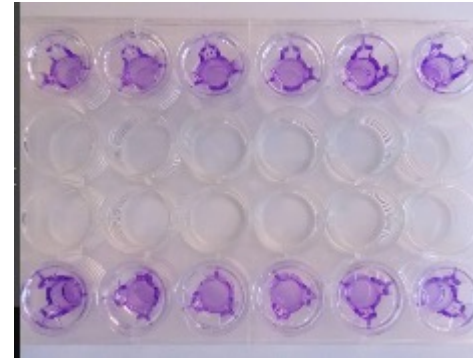
Adaptation of the models

Addition of fluidics and design of a bioreactor to mimic breathing motions

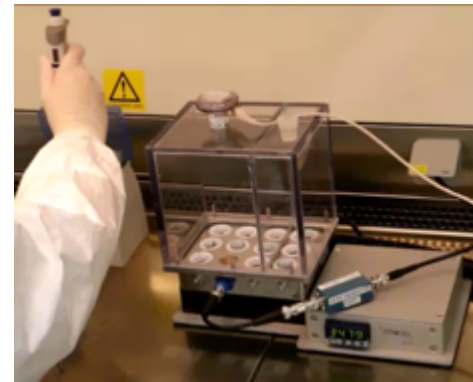


Exposures

- Concentrations used are relevant to an *in vivo* exposure ($\mu\text{g}/\text{cm}^2$)
- Exposure methods
 - Air liquid interface
 - Aerosolisation
 - Dry-powder
- Exposure scenarios
 - Acute
 - Chronic



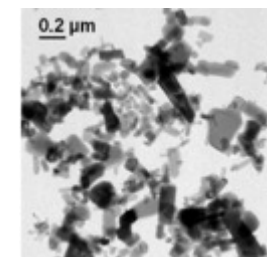
Quasi-ALI exposure



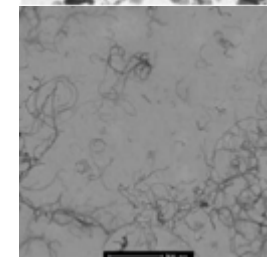
Liquid aerosol exposure
(VITROCELL® CLOUD 12)

Materials

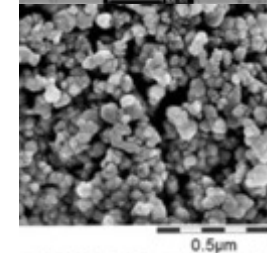
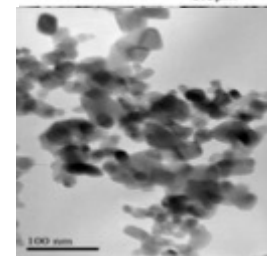
ENM Major Group ¹⁹	ENM & Supplier	Lung (inhalation)	GIT Exposure	Liver Exposure	Ecotoxicity tests
1: Soluble (release possibly toxic ions)	ZnO (NM111; JRC)	STIS & 90days- <i>Inflammation & Fibrosis</i> ³⁷	No data available	Pulmonary exposure- <i>No translocation</i>	Zebrafish larvae- <i>Developmental toxicity</i> ³⁸ ; Daphnids- <i>Accumulation and toxicity</i> ³⁹ ; <i>Algae- Toxicity</i> ⁴⁰ <i>and trophic transfer</i> ⁴¹
	Ag (Sigma 576832) (CAS#:7440-22-4) [†] or (NM300/302; JRC) [‡]	No data available	28days (Feed pellets) <i>Impact on GIT microbiota</i> ⁴²	No data available	Soil Microbe Communities <i>Structure and function toxicity</i> ⁴³
2: Biopersistent high aspect ratio ENM (fibre paradigm) ^Δ LFA will serve as a positive fibre control (WP3 only)	MWCNT ⁴⁴ (Mitsui-7)	Inhalation (2yrs); Pharyngeal aspiration (56days) ⁴⁴ <i>Fibrosis & Carcinogenicity</i>	No data available	Pulmonary exposure <i>Translocation to liver</i> ⁴⁵	No data available
	MWCNT (NM402; JRC)	STIS & 90days <i>Inflammation</i> ⁴⁶	No uptake	No data available	No data available
3: Passive (no reactivity or toxic potential)	BaSO₄ (NM220; Fraunhofer IME)	STIS, 28days, 90days, 1yr, 2yrs ^{37,47} <i>No adverse findings</i>	28days (gavage) No adverse effects ⁴⁸ .	STIS (28days) <i>Translocation to liver; no histopathology change</i> ³⁷	No data available
4: Active (positive, insoluble; promote cellular effects and/or mobility in the organism)	CeO₂ (NM212; Fraunhofer IME)	STIS, 28 & 90days, 1yr, 2yrs- <i>Inflammation</i> ⁴⁹	90days (feed pellets)	STIS (28days) <i>Translocation to liver; no histopathology</i> ⁴⁹	Algae and Daphnids <i>Chronic toxicity</i> ^{50,51}
	TiO₂ (NM105; JRC) [*]	STIS, 90days, 2yrs exposures ⁵² <i>Inflammation</i> Carcinogenicity study (currently ongoing by JBRC)	No data available	No data available	Zebrafish larvae- <i>Developmental toxicity</i> ⁵³ ; Daphnids- <i>Toxicity</i> ⁵⁴ ; <i>Algae- Toxicity and trophic transfer</i> ⁴¹
	Amorphous SiO₂ (SAS; IUF)	No data available	28 & 84days (mice, feed pellets); 84days (rats, feed) <i>Fibrosis</i> ⁵⁵	No data available	No data available
	Crystalline SiO₂ [‡] (DQ ₁₂ quartz; IOM) [*]	STIS & 2yrs exposures <i>Inflammation</i>	No data available	No data available	No data available



ZnO

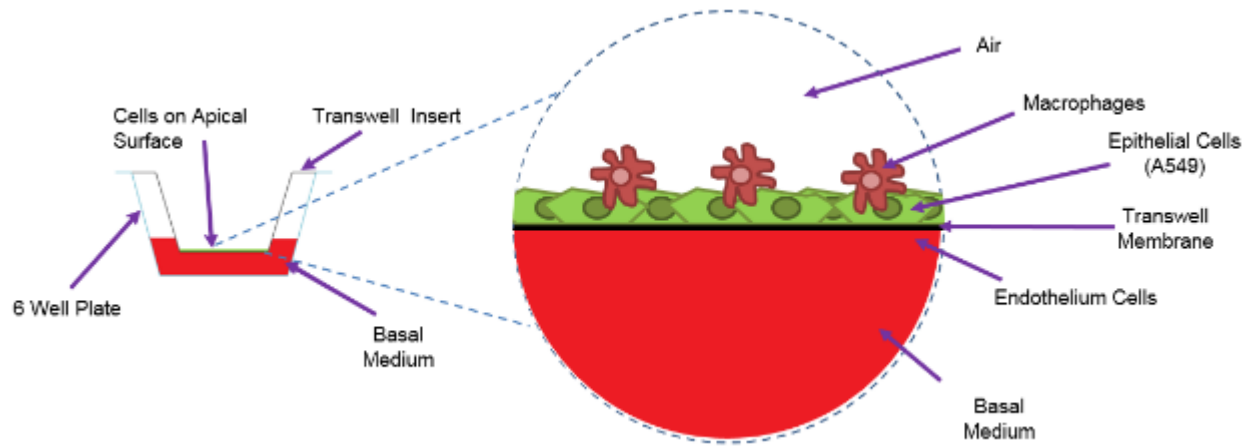


MWCNT

BaSO₄TiO₂

Lung Models

Inflammatory lung model



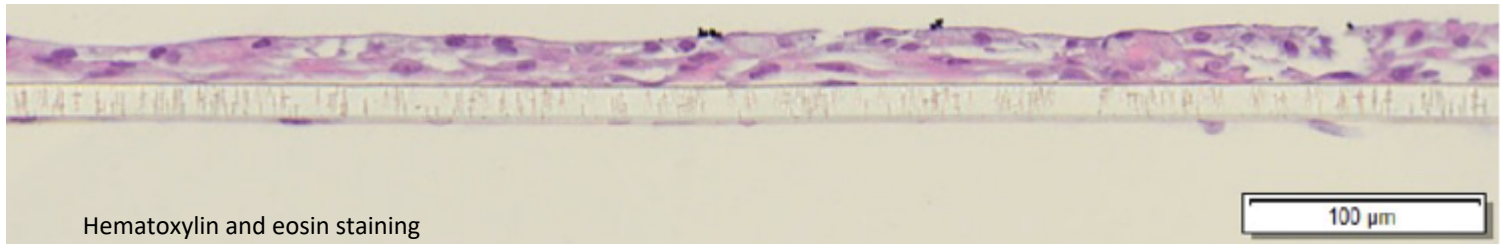
Fibrotic lung model

Primary co-culture system: EpiAlveolar™



Patrick Hayden
Anna G. Maione

Macrophages
Epithelial cells
Fibroblasts
Endothelial cells



AOP



Summary

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

Acknowledgments



PATROLS Partners



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

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