PATROLS

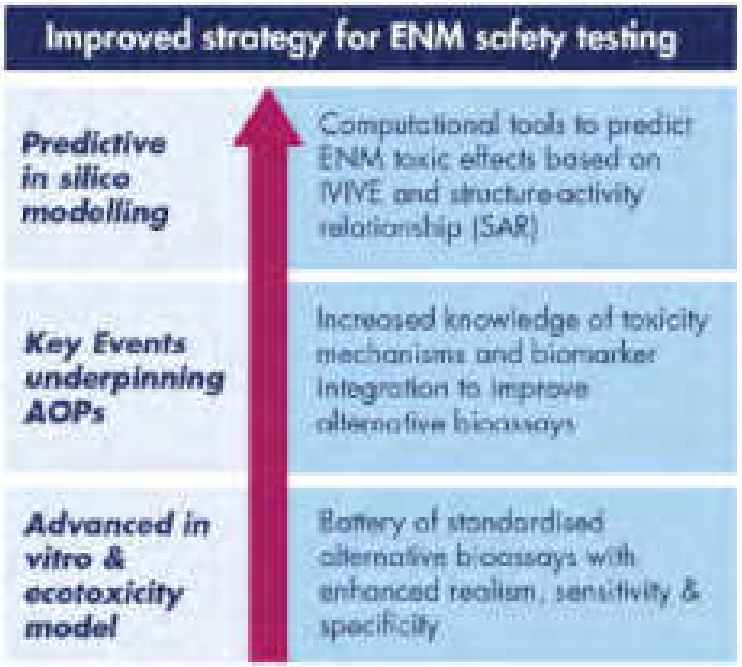
Advanced Tools for NanoSafety Testing

# Physiologically Anchored Tools for Realistic nanOmaterial hazard aSsessment

Barbara Rothen-Rutishauser, Adolphe Merkle Institute, University Fribourg, Switzerland

# 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)

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# PATROLS Concept

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# WP3 - Advanced in vitro pulmonary models for ENM hazard assessment

- **AMI:** Barbara Rothen-Rutishauser, Hana Barosova, Anne Bannuscher
- **SU:** Martin Clift, Kirsty Meldrum
- **HWU:** Vicki Stone, David Brown
- **RIVM:** Hedwig Braakhuis, Rob Vandebriel
- **MISVIK:** Roland Grafström, Vesa Hongisto, Penny Nymark
- **UNIPI:** Arti Ahluwalia, Roberta Nossa
- **LTAP:** Sybille van den Brule, Dominique Lison
- **BASF:** Lan Ma-Hock, Barbara Birk

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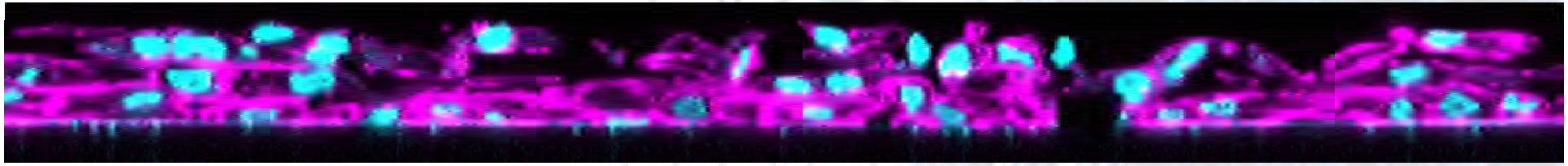
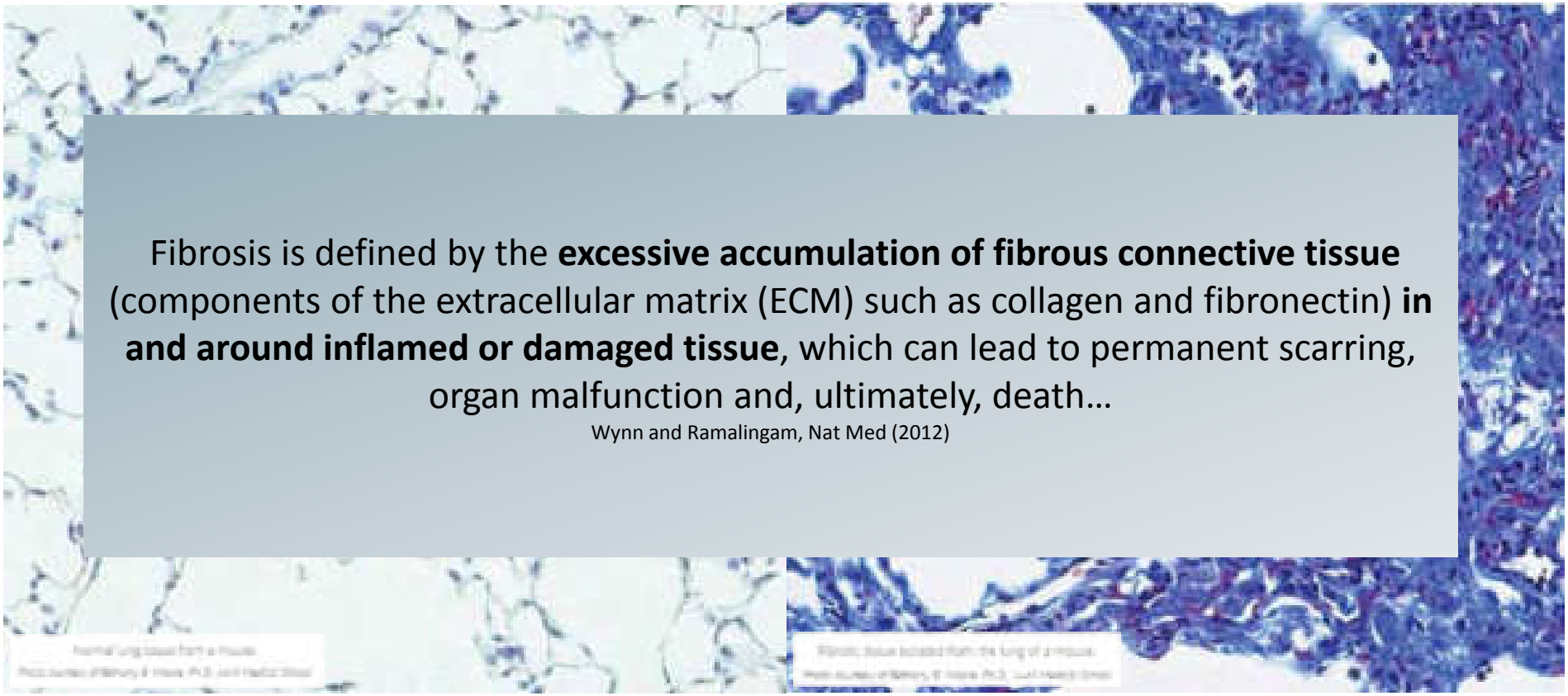
# *In vitro* tests for lung fibrosis KEs



# The pathogenesis of pulmonary fibrosis

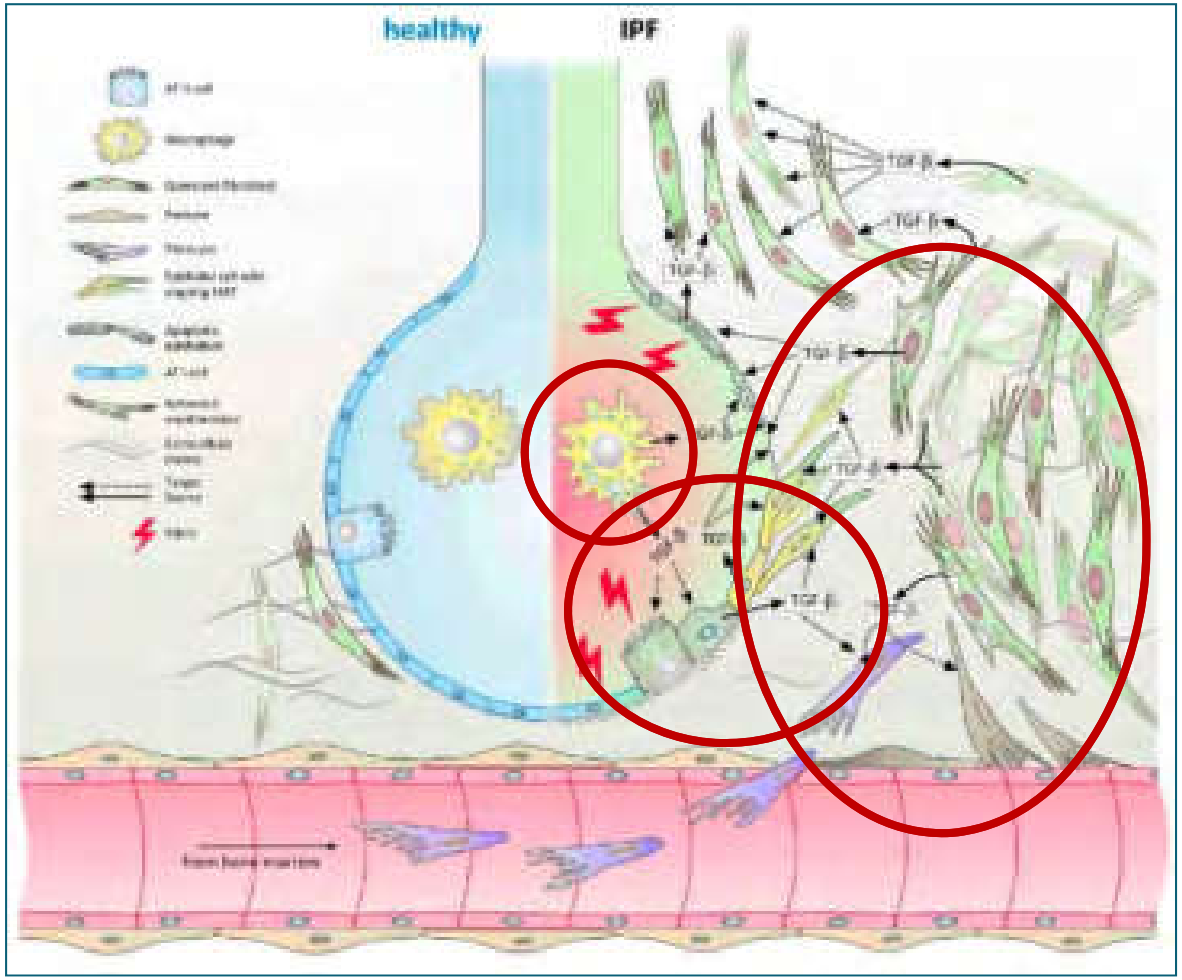


Fibrosis is defined by the **excessive accumulation of fibrous connective tissue** (components of the extracellular matrix (ECM) such as collagen and fibronectin) **in and around inflamed or damaged tissue**, which can lead to permanent scarring, organ malfunction and, ultimately, death…

Wynn and Ramalingam, Nat Med (2012)

https://www.britannica.com/science/pulmonary-fibrosis

# Adverse outcome pathway (AOP) framework for pulmonary fibrosis




Fernandez and Eickelberg, Proc Am Thorac Soc 2012

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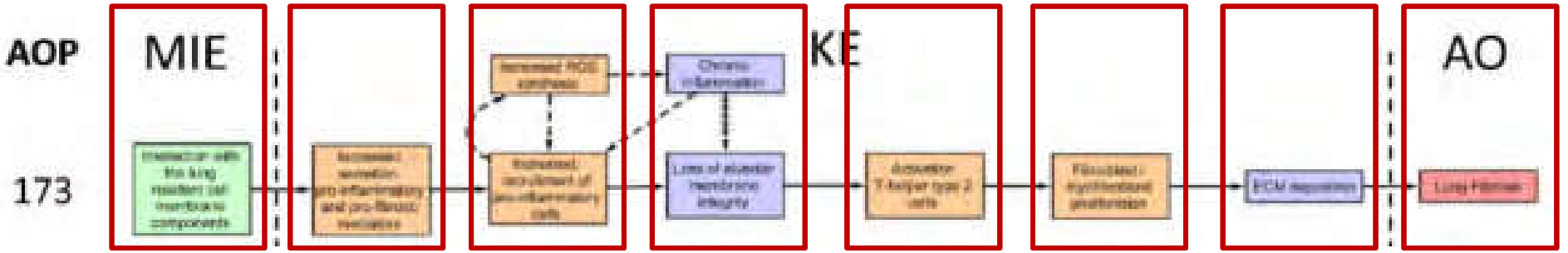
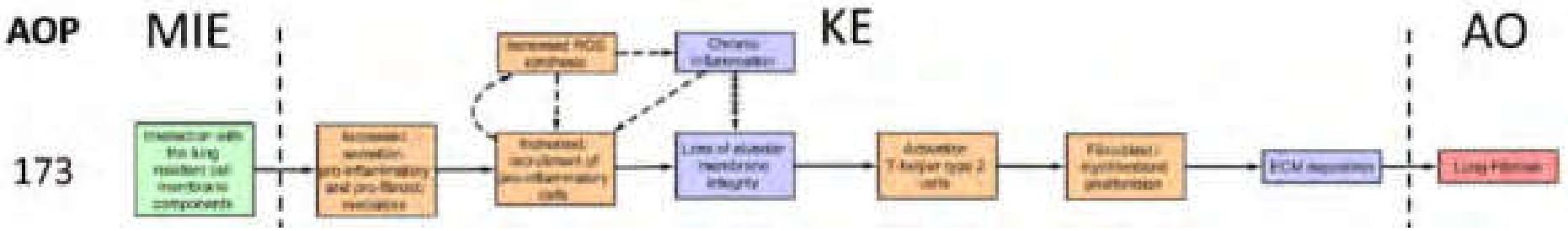



https://aopwiki.org/aops/173; Halappanavar et al. Part Fibre Toxicol (2020)

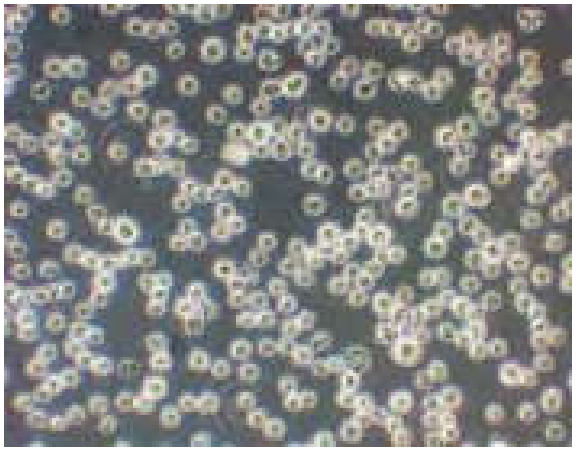
# Choice of relevant lung cell models / (nano)materials / endpoint(s)



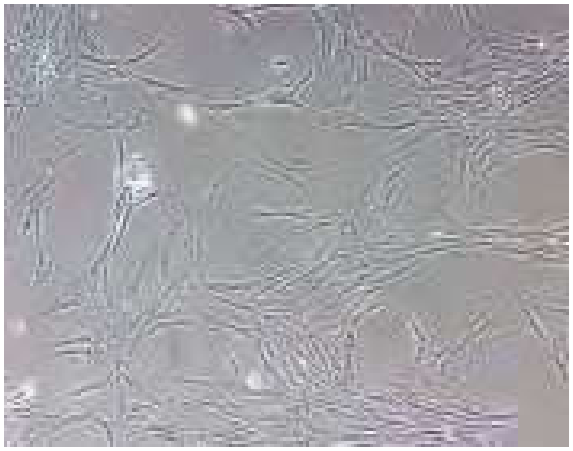

https://aopwiki.org/aops/173; Halappanavar et al. Part Fibre Toxicol (2020)

# Choice of relevant lung cell models / (nano)materials / endpoint(s)

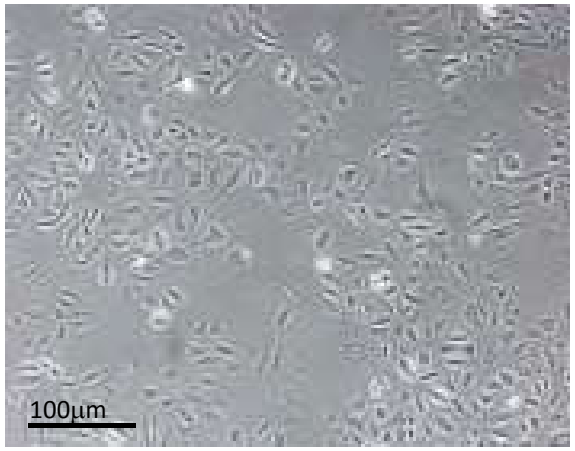
Human **macrophages**



Human **fibroblasts**



Human **alveolar epithelial cells**




Drasler et al. NanoImpact 8 (2017)

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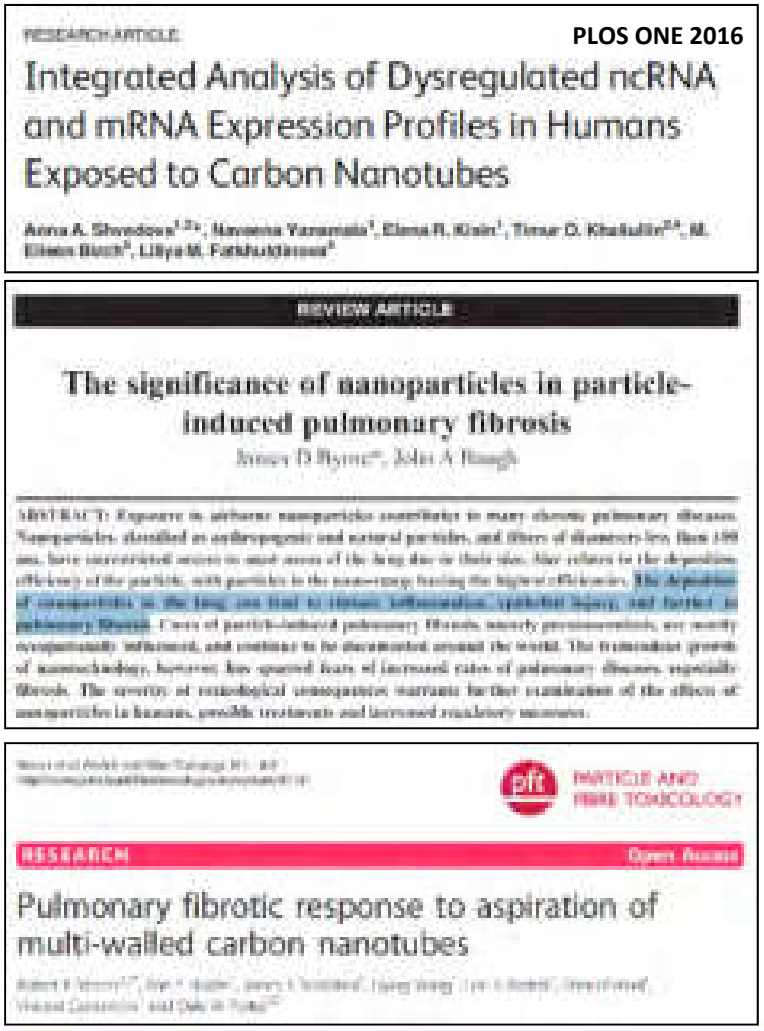


PLOS ONE 2016

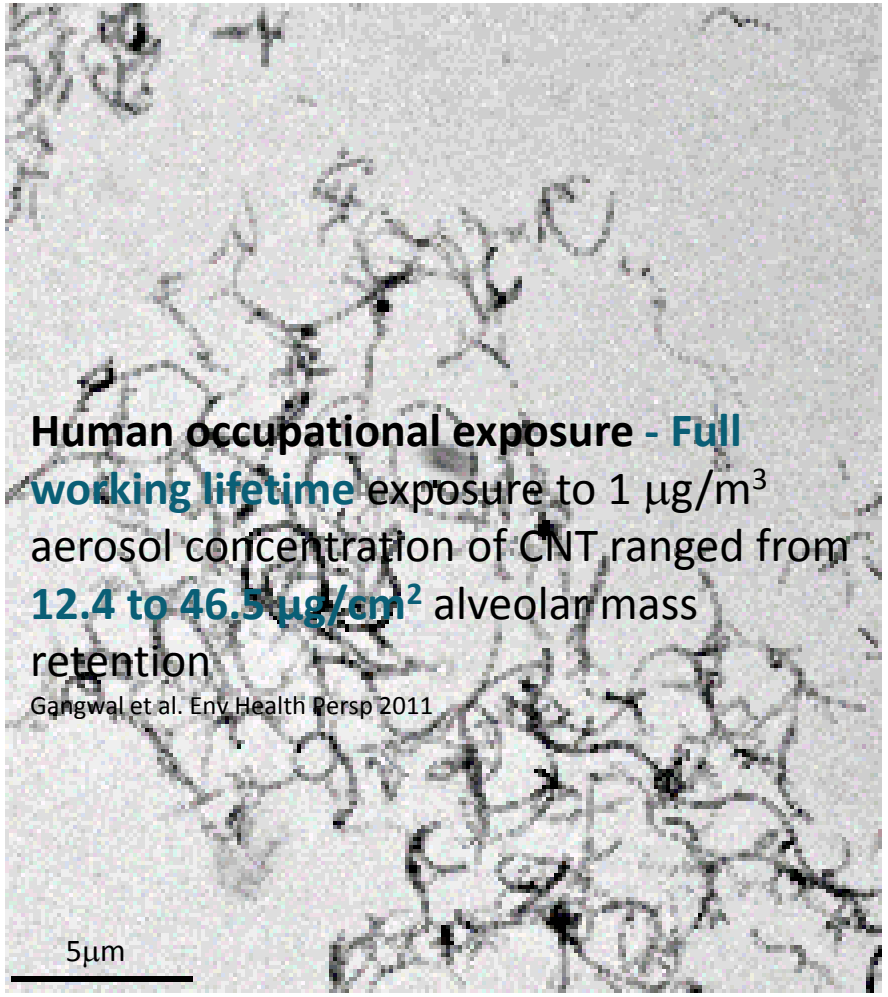
Integrated Analysis of Dysregulated ncRNA and mRNA Expression Profiles in Humans Exposed to Carbon Nanotubes

REVIEW ARTICLE

The significance of nanoparticles in particle-induced pulmonary fibrosis

RESEARCH

Pulmonary fibrotic response to aspiration of multi-walled carbon nanotubes




**Human occupational exposure - Full working lifetime** exposure to 1 µg/m$^3$ aerosol concentration of CNT ranged from **12.4 to 46.5 µg/cm$^2$** alveolar mass retention

Gangwal et al. Env Health Persp 2011

Chortarea et al. ACS Nano 2017

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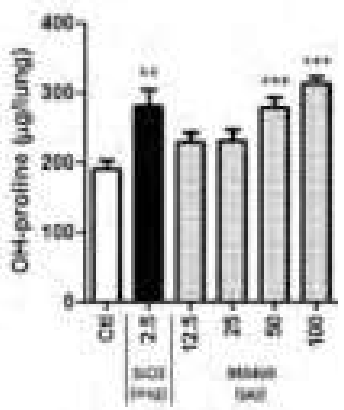
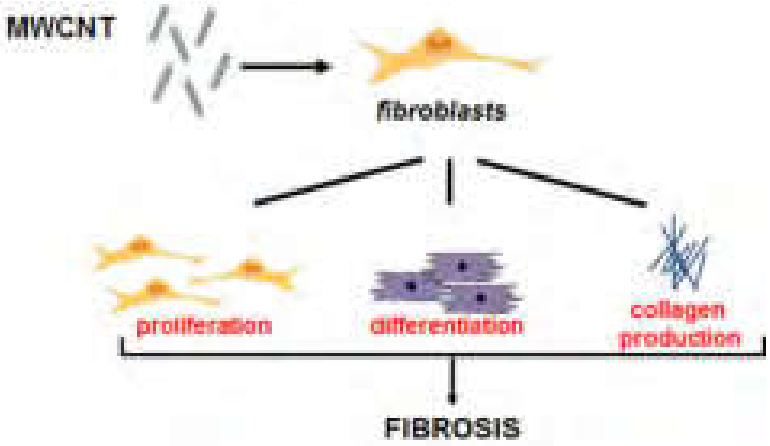
Dominique Lison
Sybille van den Brule

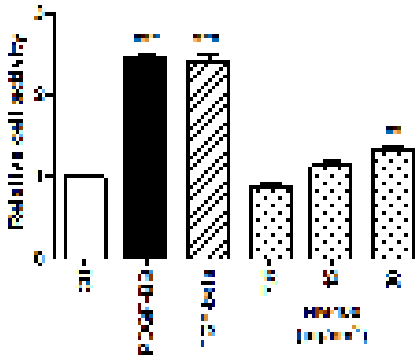
# CNTs stimulate the proliferation of fibroblasts *in vitro*

***In vivo***

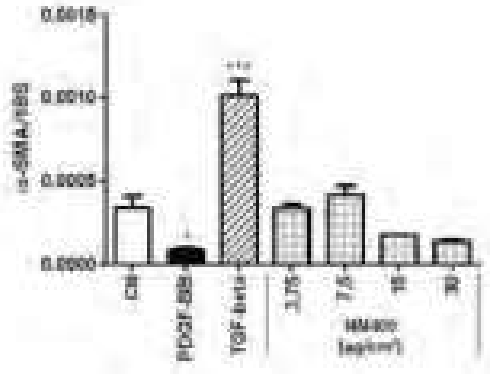


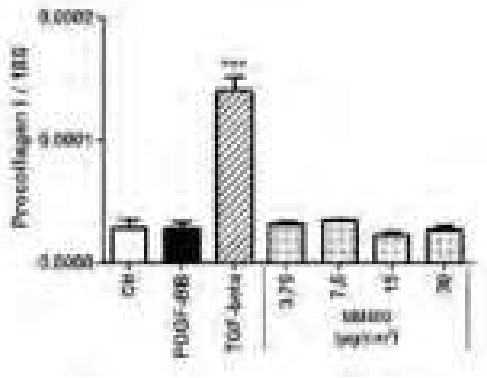

**Proliferation**



**Differentiation**



**Collagen production**

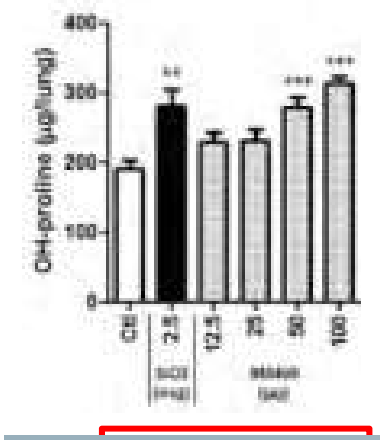


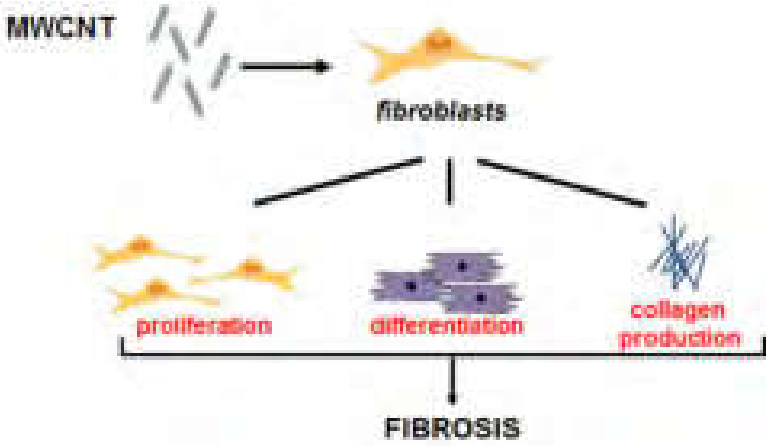
Vietti et al. Part Fibre Toxicol (2013)

Dominique Lison
Sybille van den Brule

# CNTs stimulate the proliferation of fibroblasts *in vitro*

***In vivo***



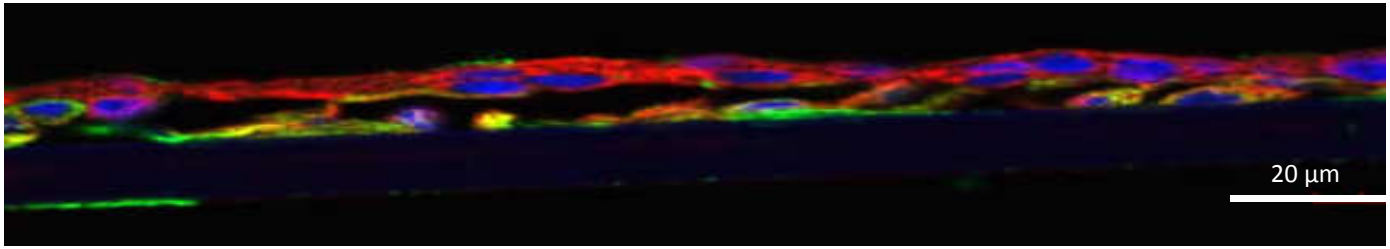




- The *in vitro* proliferative activity of CNT reflects the *in vivo* fibrosis findings, supporting a **predictive value of the *in vitro* assay**
- The **structure/length of CNT** constitute an important physicochemical determinant in the capacity of these materials to induce lung fibrosis

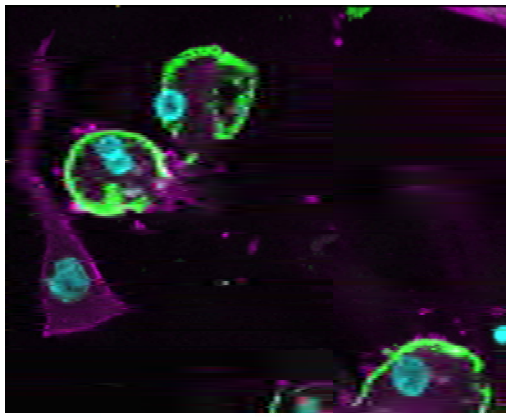
Vietti et al. Part Fibre Toxicol (2013)

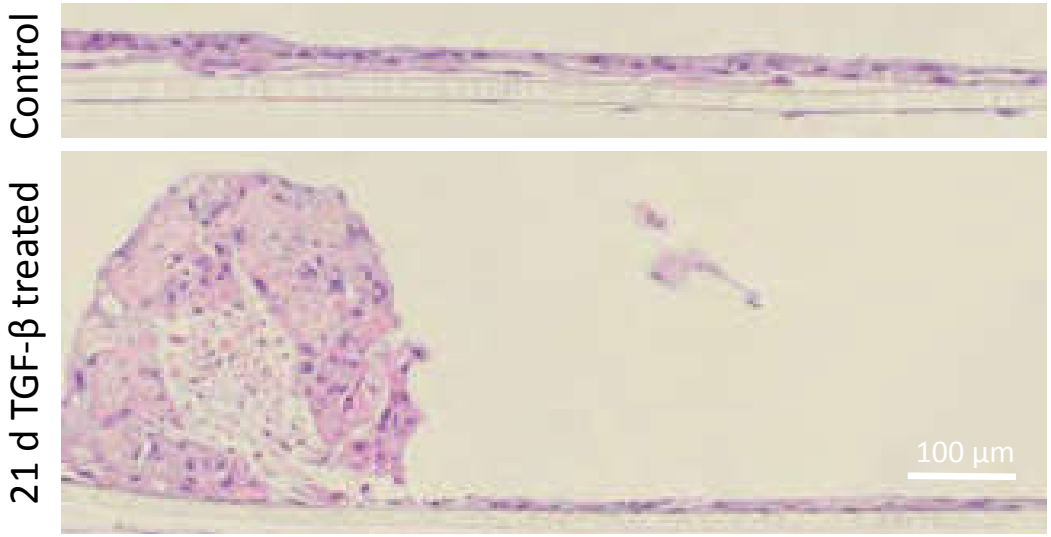
# EpiAlveolar™ lung model to predict fibrosis

Nuclei
Epithelial cells
Fibroblasts
Endothelial cells



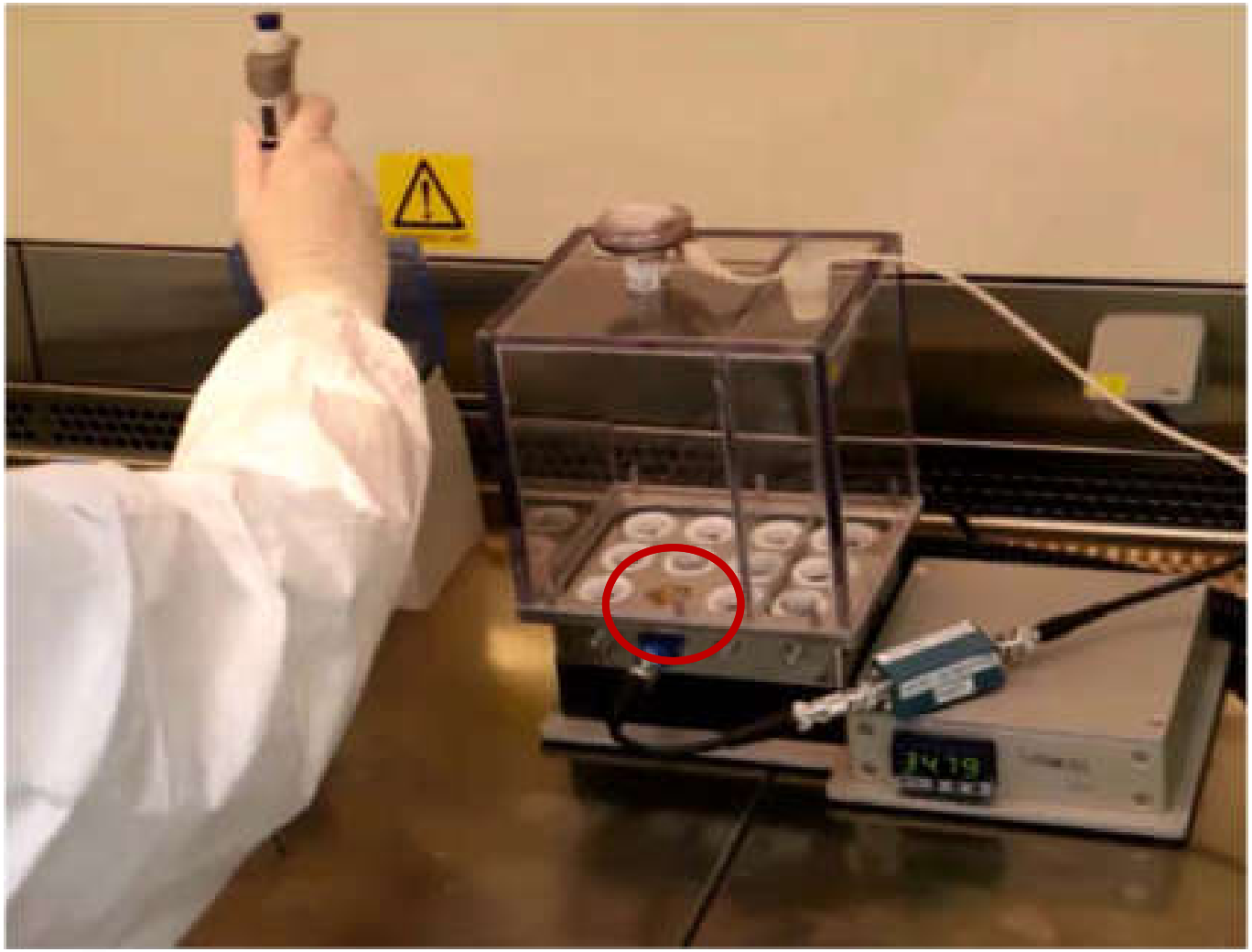







Barosova et al. ACS Nano (2020)

# Repeated (nano)material exposures



S. Chortarea, H. Barosova

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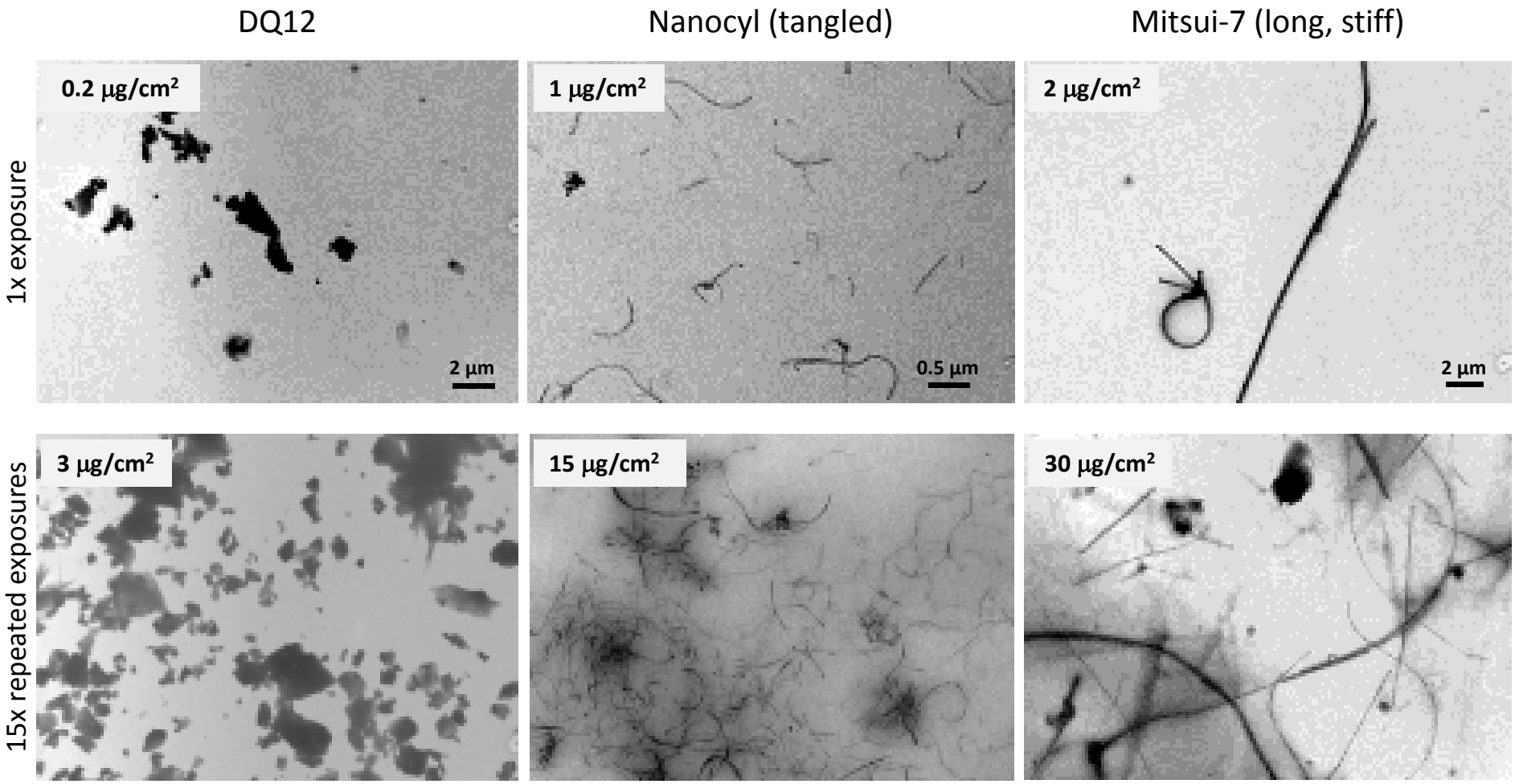
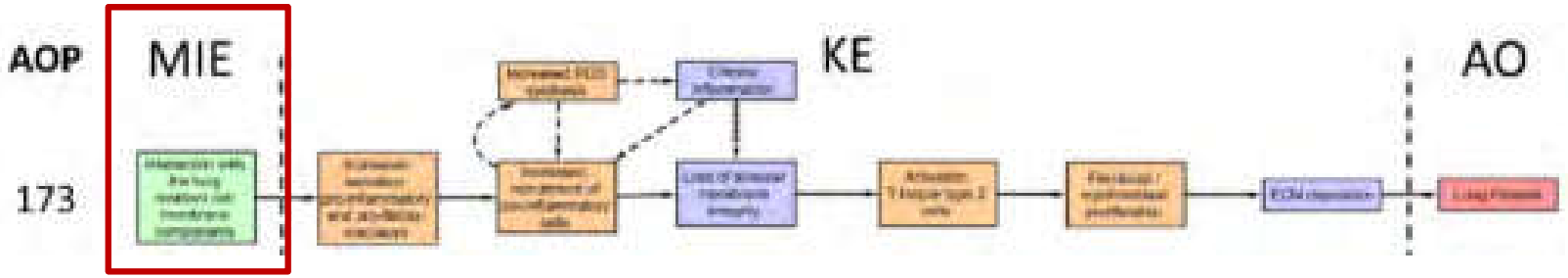
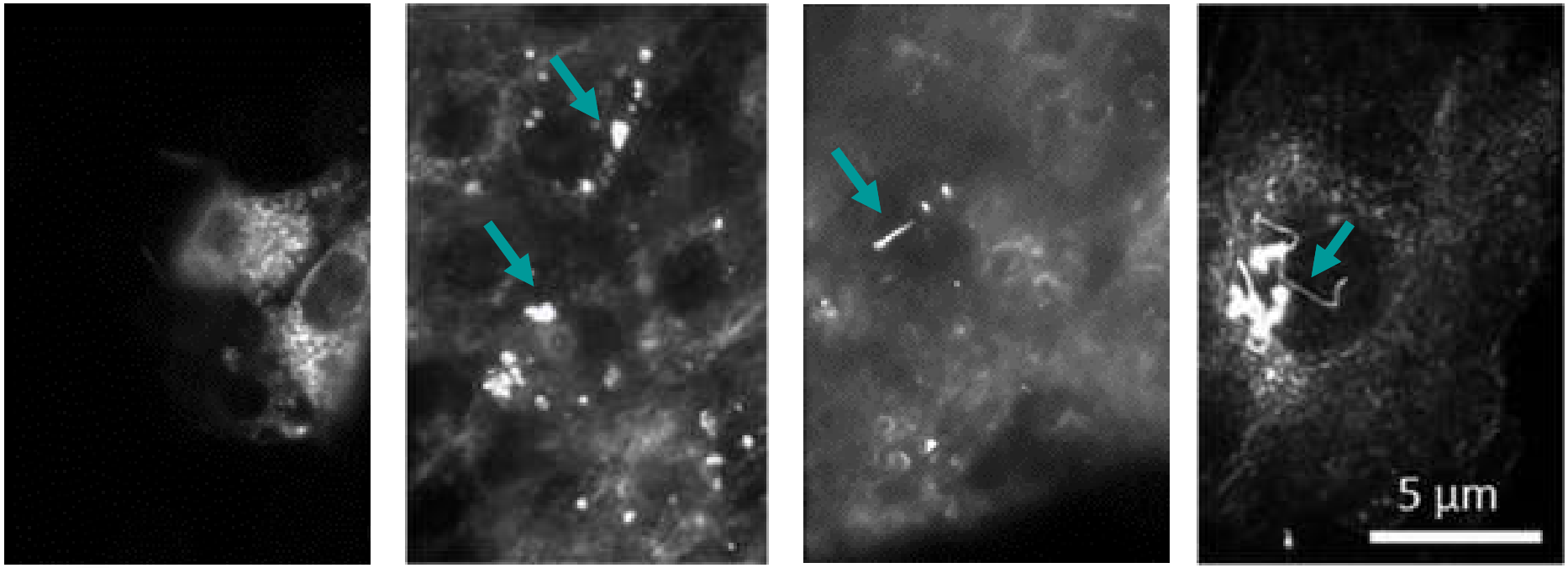



Barosova et al. ACS Nano (2020)

# Repeated (nano)material exposures to EpiAlveolar™ cells




Control | DQ12 | Nanocyl (tangled) | Mitsui-7




Barosova et al. ACS Nano (2020)

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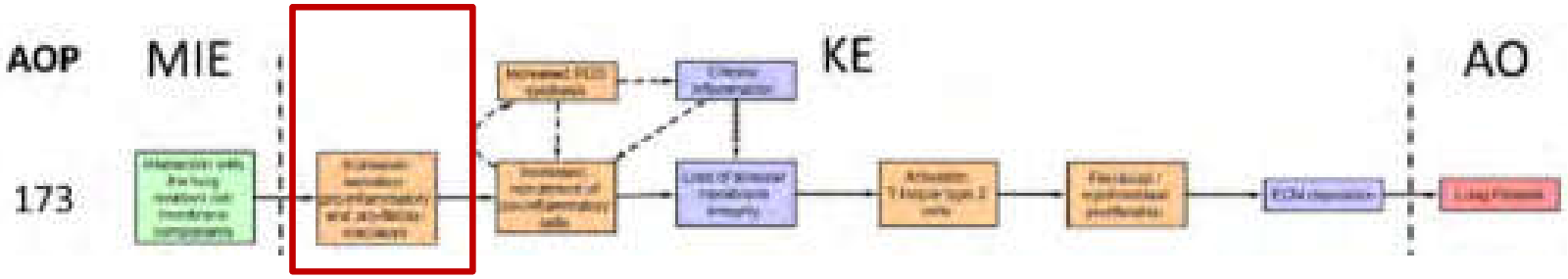


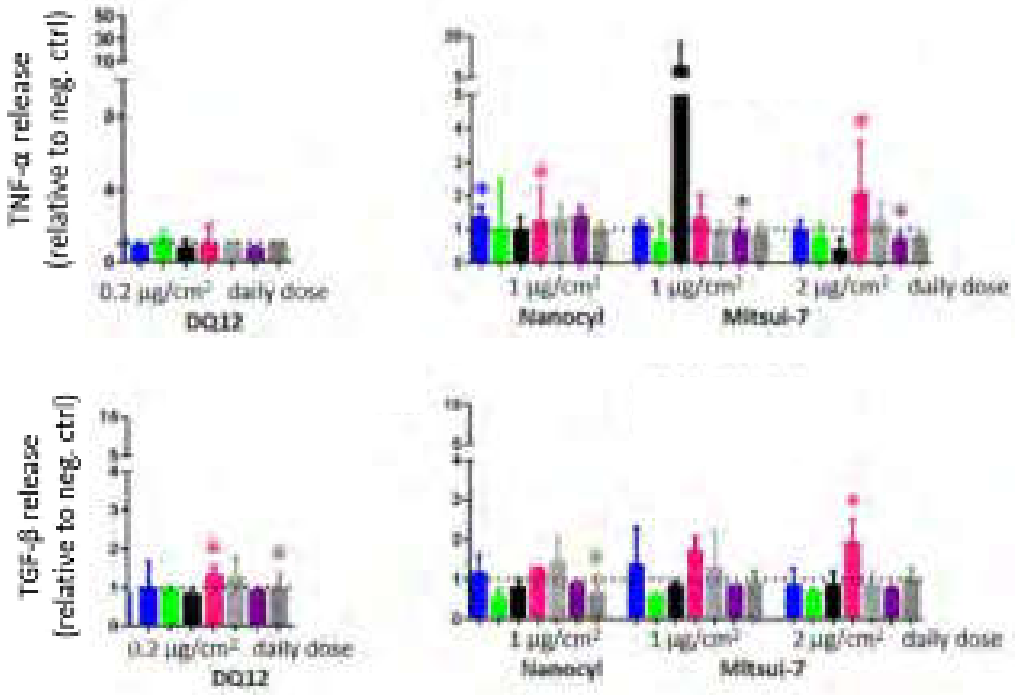


Barosova et al. ACS Nano (2020)

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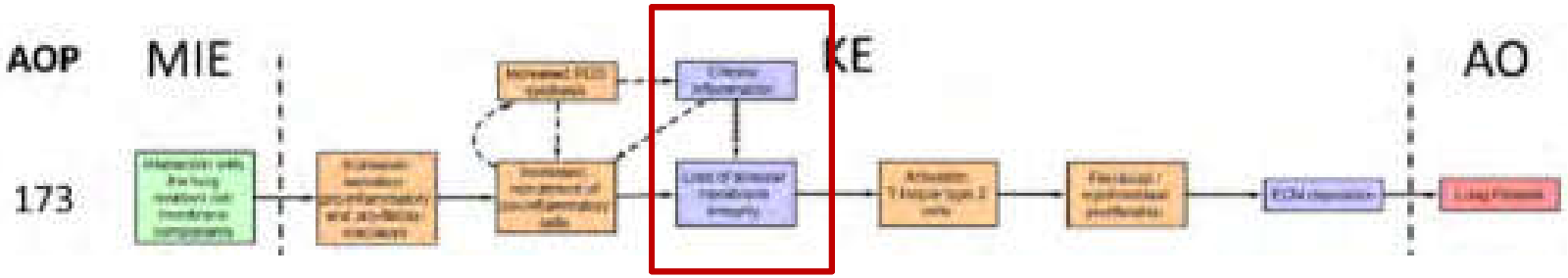
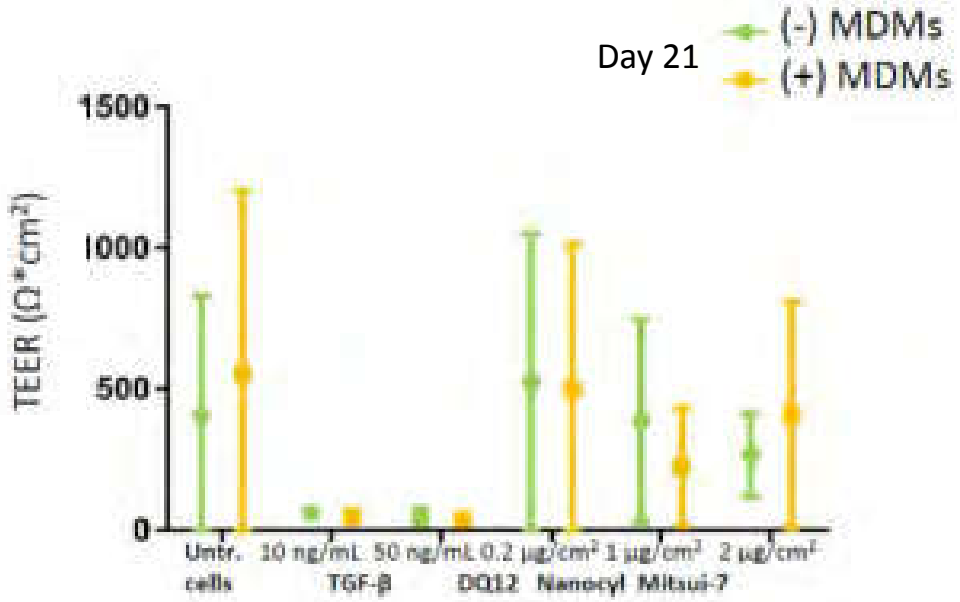
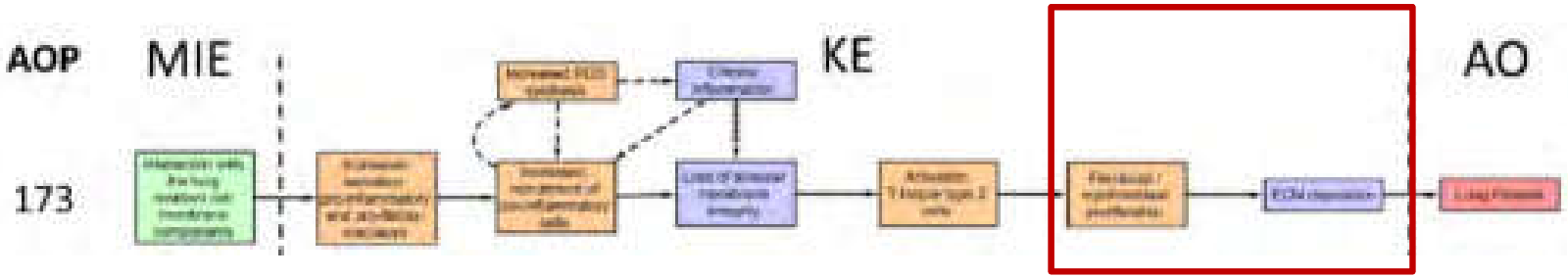
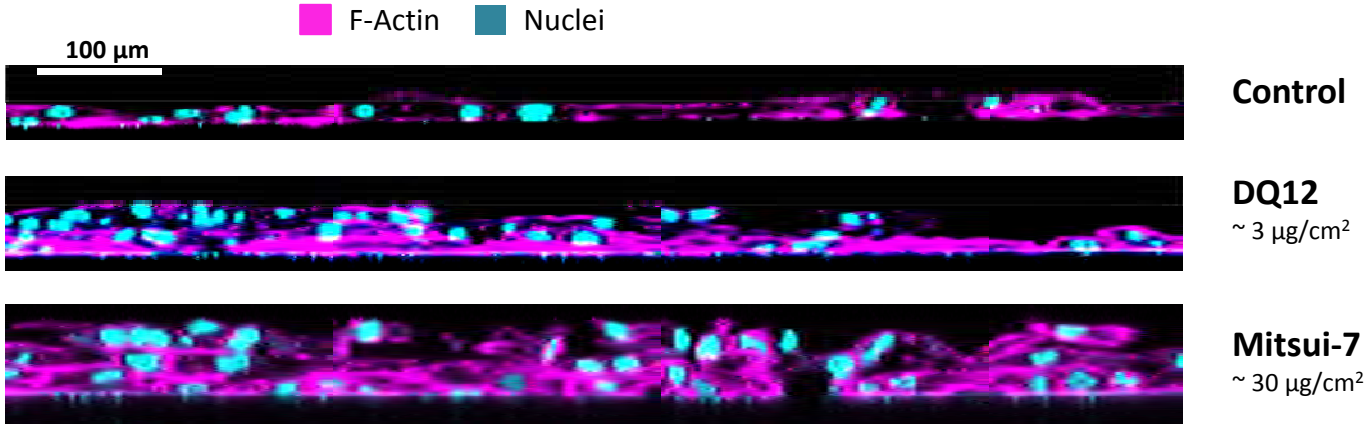



Barosova et al. ACS Nano (2020)

# Repeated (nano)material exposures to EpiAlveolar™ cells

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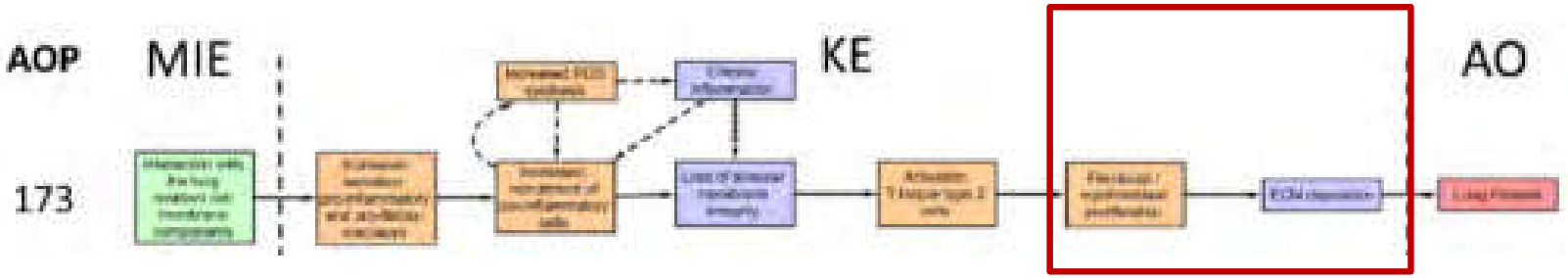
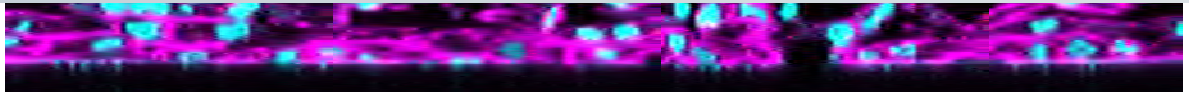



F-Actin Nuclei

100 µm

- The **EpiAlveolar™ model** can **predict inflammatory and fibrotic responses** upon repeated exposure to **aerosolized carbon nanotubes and DQ12**



**Mitsui-7**
~ 30 µg/cm²

Barosova et al. ACS Nano (2020)

…3D epithelial tissue models to predict fibrosis…

Definition: ***If you make a prediction about something, you say what you think will happen.***

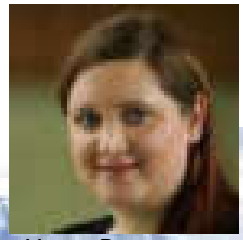
https://www.collinsdictionary.com/dictionary/english/prediction

**3D epithelial tissue models**

- Structural-functional characterisation
- Relevant endpoints, AOP concept (positive controls!)
- Dosimetry (Air-liquid exposure systems)
- Reliability / Reproducibility

Understanding the process and interaction of the various stakeholders of the **standardization**, **validation**, and **approval procedure** for an **alternative test method**

## BioNanomaterials group



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Adolphe Merkle Foundation

University of Fribourg

Collaboration partners:

- P. Gehr / F. Blank, University of Bern
- O. Schmid, Helmholtz Center Munic
- M. Clift, Swansea University
- MatTek Corporation
- Peta International Science Consortium
- V. Stone, Heriot-Watt University

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