



PATROLS

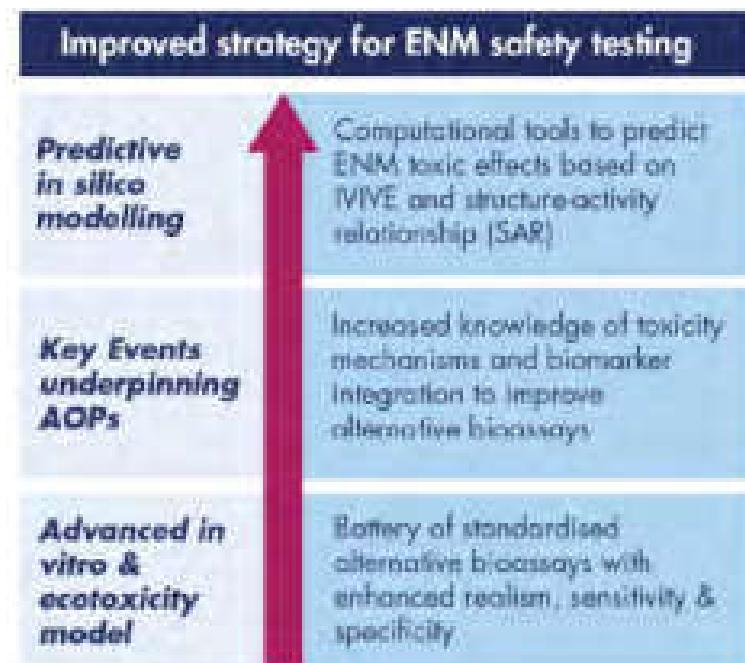
Advanced Tools for NanoSafety Testing

Physiologically Anchored Tools for Realistic nanOmateriaL hazard aSsessment

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

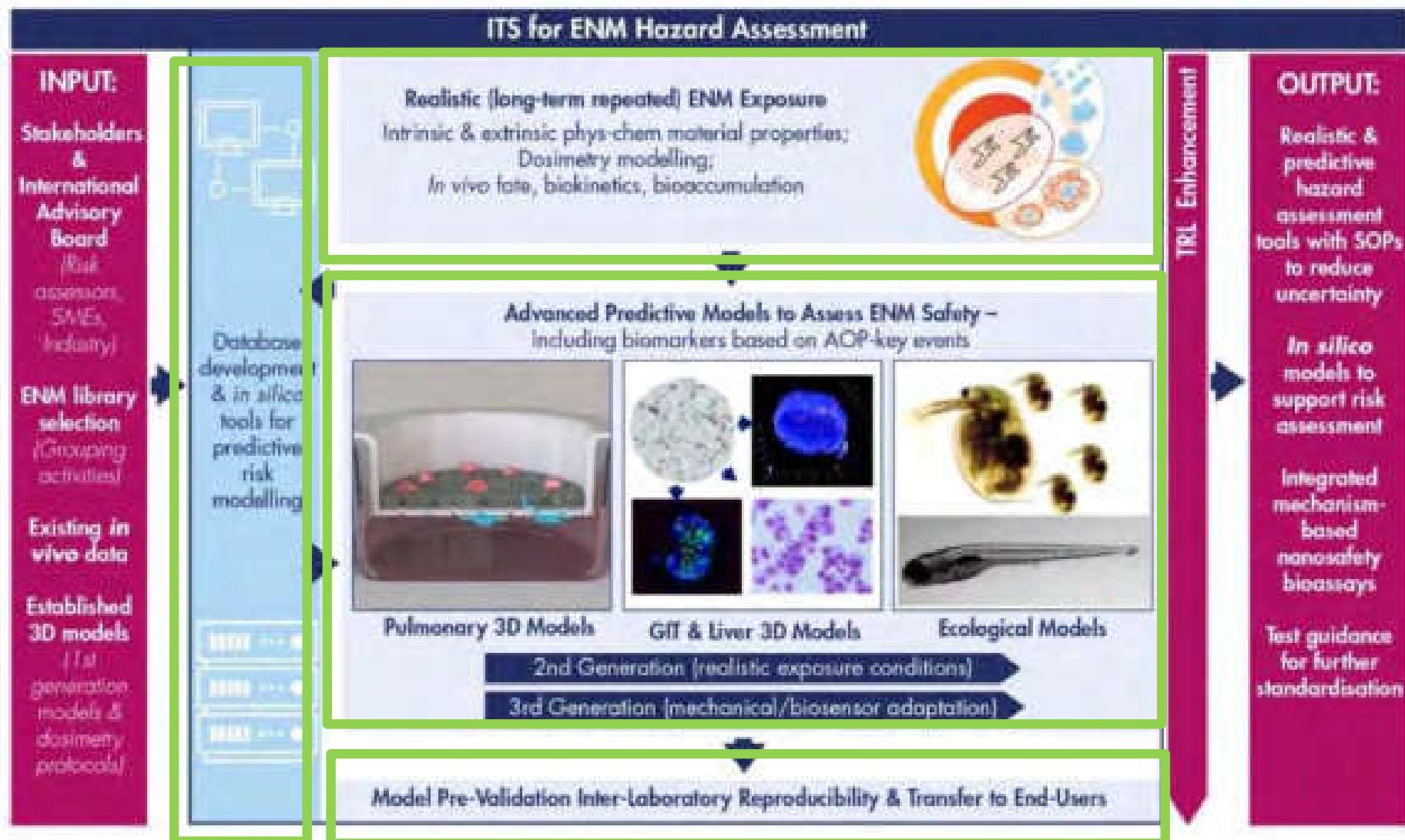
PATROLS aim & vision

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)

PATROLS Concept



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



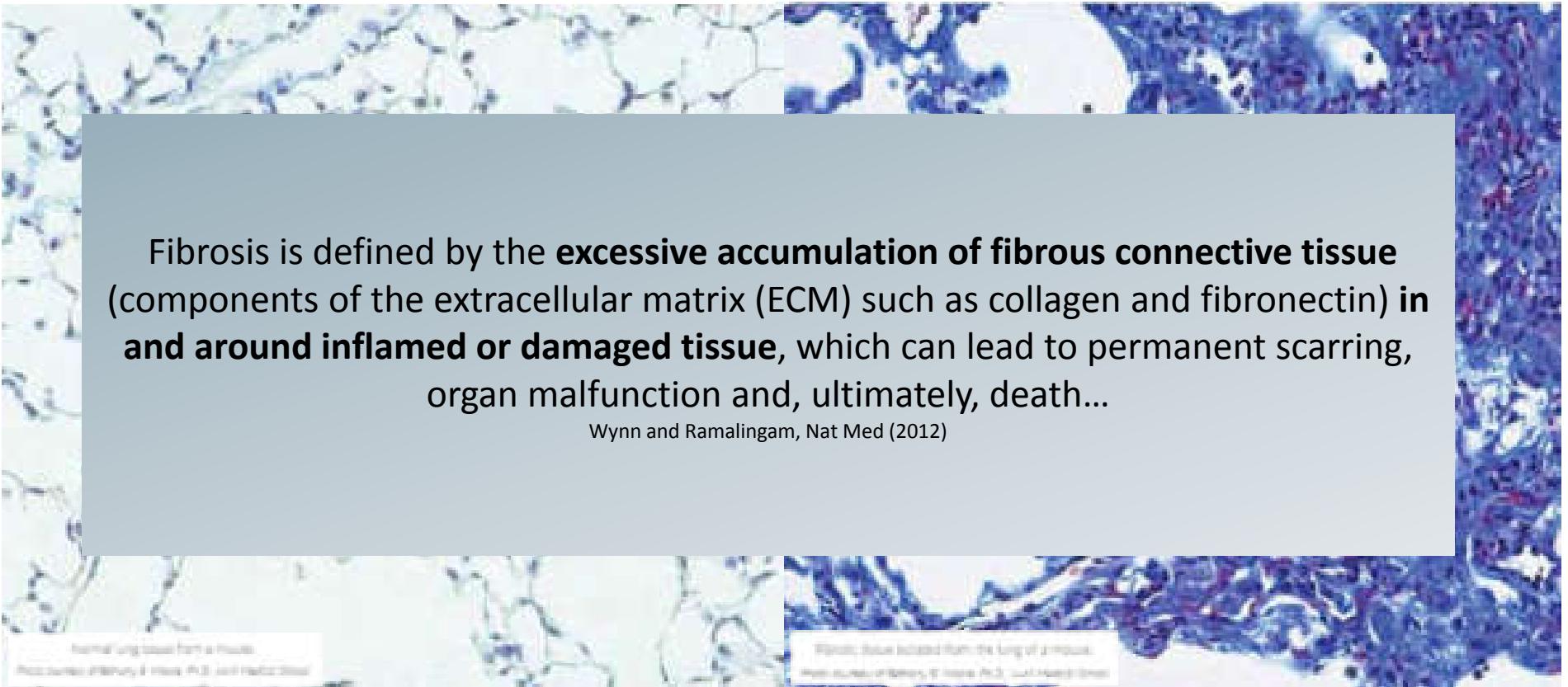
PATROLS

Advanced Tools for NanoSafety Testing

In vitro tests for lung fibrosis KEs



The pathogenesis of pulmonary fibrosis

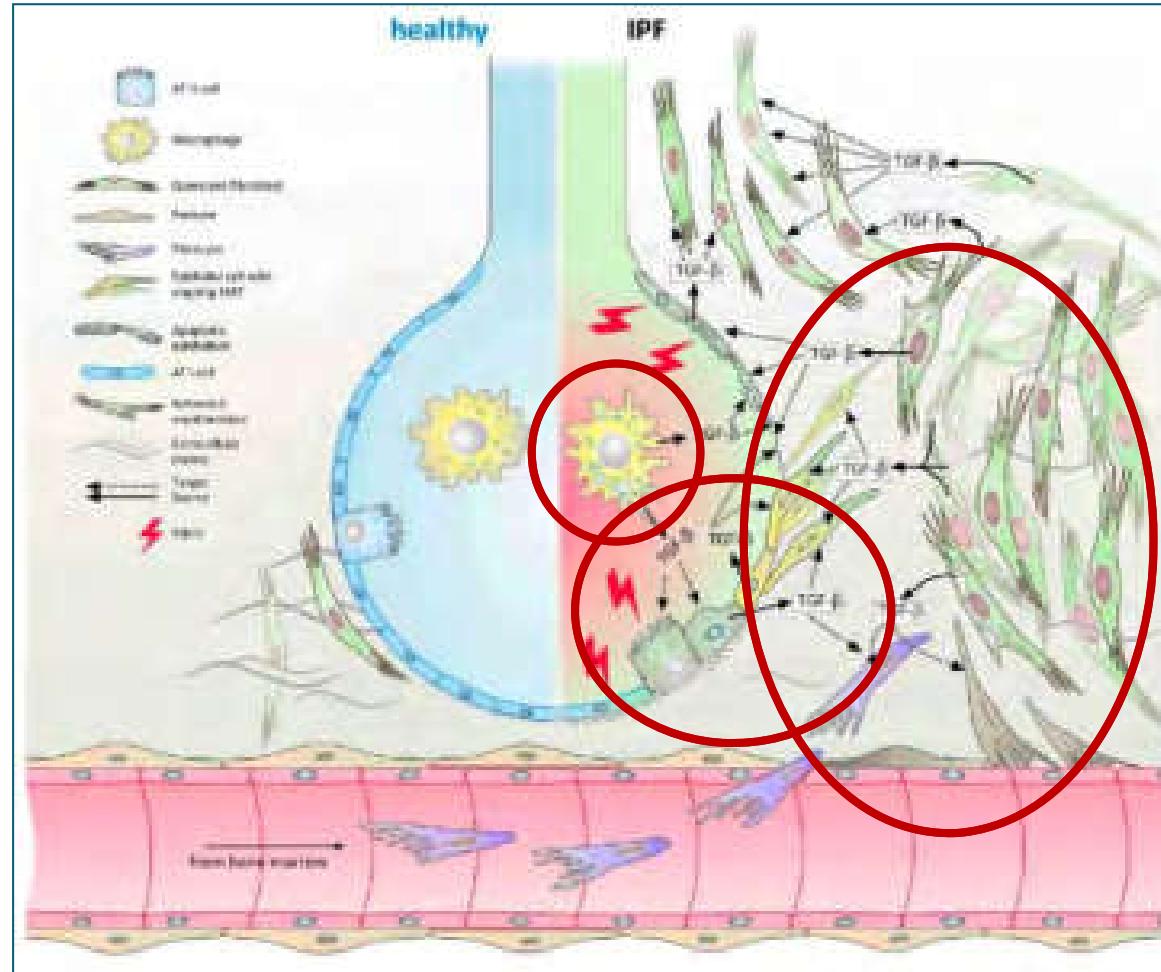


Fibrosis is defined by the **excessive accumulation of fibrous connective tissue** (components of the extracellular matrix (ECM) such as collagen and fibronectin) **in and around inflamed or damaged tissue**, which can lead to permanent scarring, organ malfunction and, ultimately, death...

Wynn and Ramalingam, Nat Med (2012)

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

Adverse outcome pathway (AOP) framework for pulmonary fibrosis



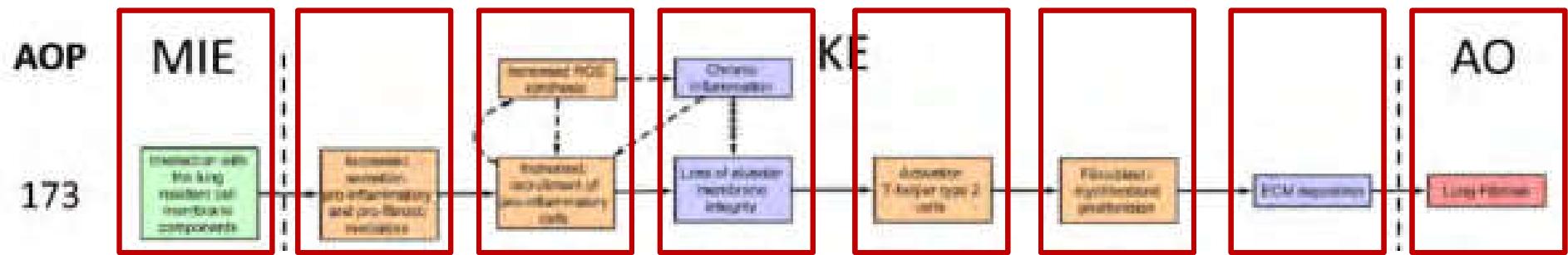
Fernandez and Eickelberg, Proc Am Thorac Soc 2012



PATROLS

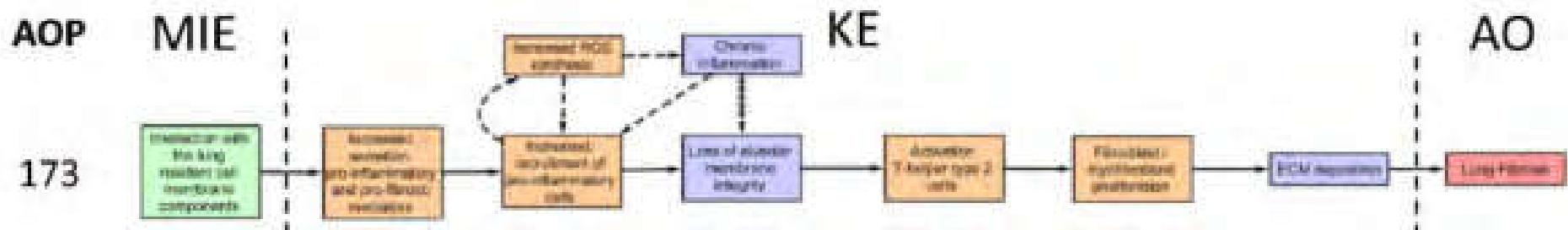
Advanced Tools for NanoSafety Testing

Adverse outcome pathway (AOP) framework for pulmonary fibrosis



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

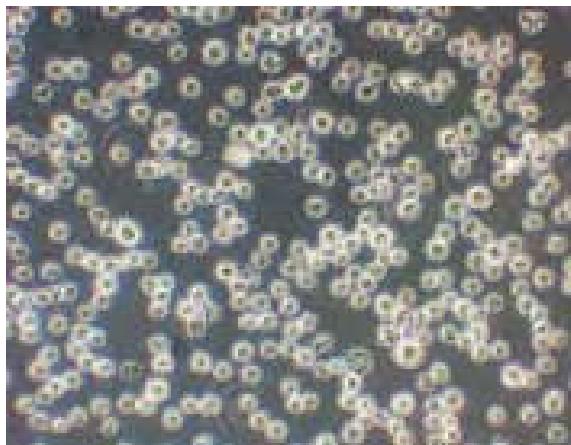
Choice of relevant lung cell models / (nano)materials / endpoint(s)



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

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

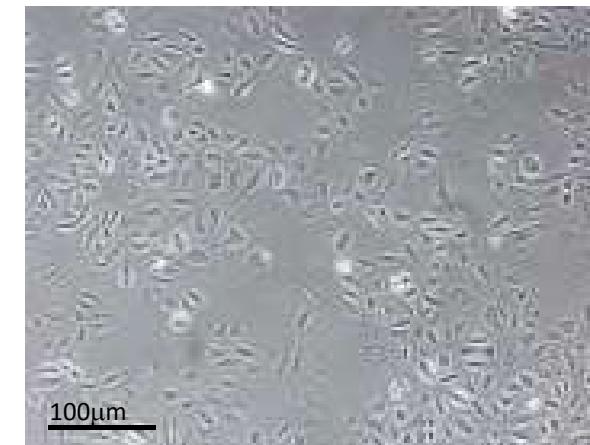
Human macrophages



Human fibroblasts



Human alveolar epithelial cells



Drasler et al. NanoImpact 8 (2017)

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

RESEARCH ARTICLE

PLOS ONE 2016

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

Anna A. Shvedova^{1,2*}, Navaneeth Venkatesh¹, Elena R. Klein¹, Tinsur O. Khalilov^{2,3}, M. Elham Bush², Liliya M. Pashutina^{2,3}

REVIEW ARTICLE

The significance of nanoparticles in particle-induced pulmonary fibrosis

James D Byrne^a, John A Haug^b

INTRODUCTION Exposure to airborne nanoparticle aerolites in many chronic pulmonary diseases. Nanoparticles, classified as anthropogenic and natural particles, and fibers of diameter less than 100 nm, have concentrated access to most areas of the lung due to their size. Also relates to the deposition efficiency of the particles, with particles in the nanosizing having the highest efficiency. The deposition of nanoparticles in the lung can lead to human inflammation, epithelial injury, and further to pulmonary fibrosis. Cases of particulate-induced pulmonary fibrosis, mainly pneumoconioses, are mostly occupationally influenced, and continue to be disseminated around the world. The tremendous growth of nanotechnology, however, has spurred fears of increased rates of pulmonary diseases, especially fibrosis. The severity of pathophysiological consequences warrants further evaluation of the effects of nanoparticles in humans, possible treatments and increased regulatory measures.

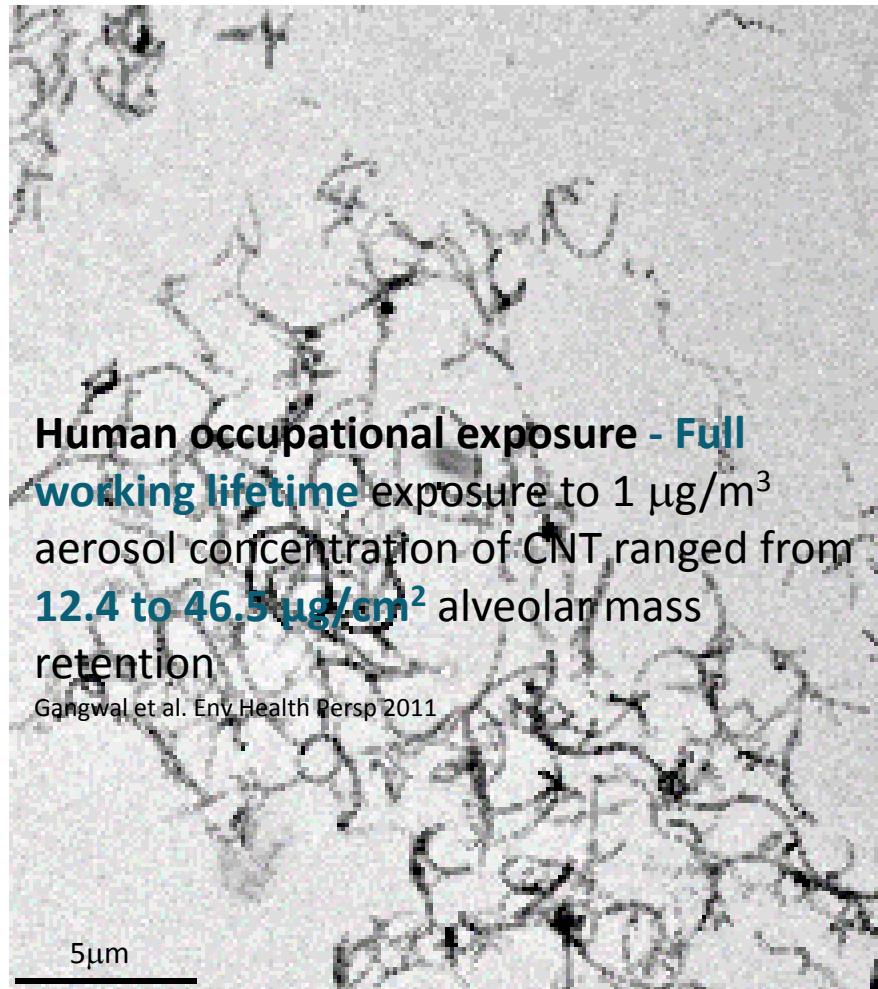
Journal of Applied Toxicology 31: 1–10
https://doi.org/10.1002/jat.3119

PARTICLE AND FIBER TOXICOLOGY

RESEARCH Open Access

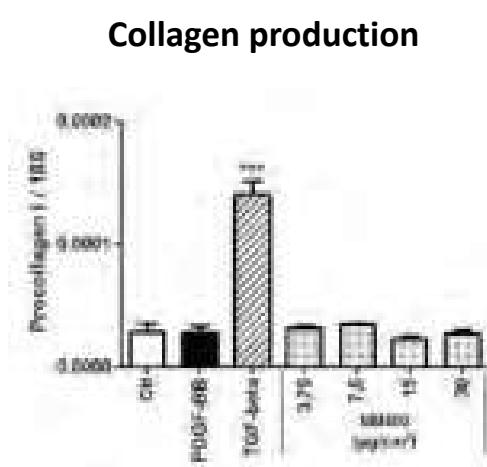
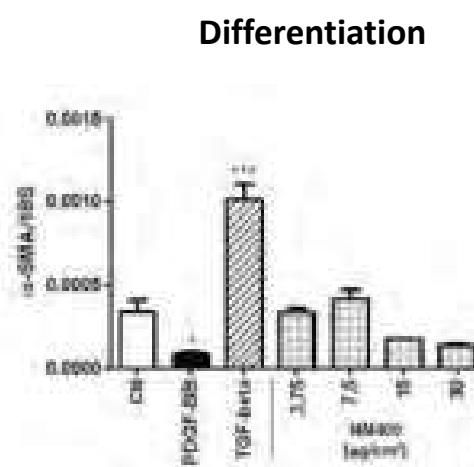
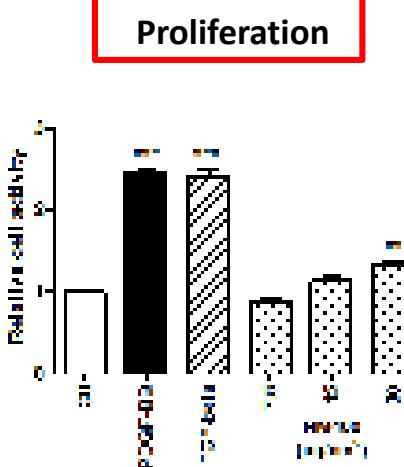
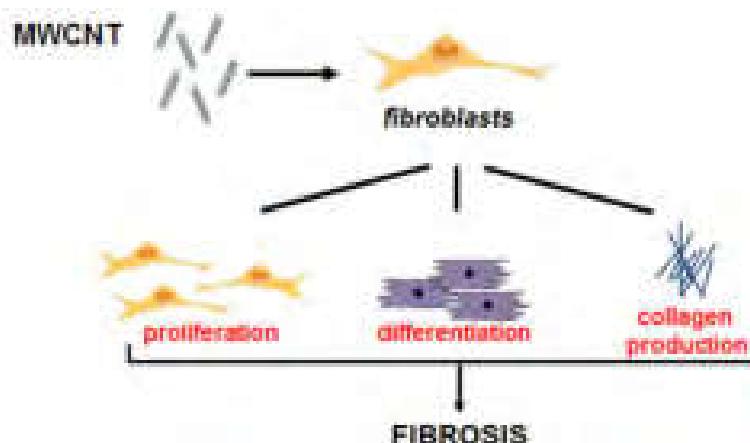
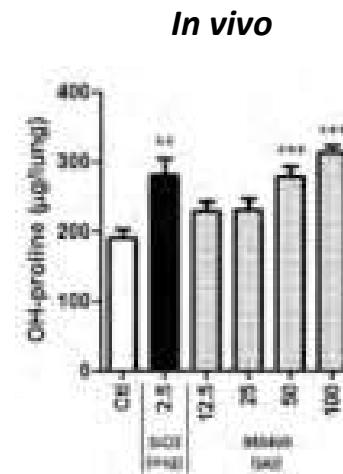
Pulmonary fibrotic response to aspiration of multi-walled carbon nanotubes

Robert J. Murray^{1,2}, Sean C. Hsu³, James C. Hunter⁴, Liang Wang⁵, Lee J. Hwang⁶, David J. Gitter⁷, Michael J. Campbell⁸, and Dale W. Price⁹

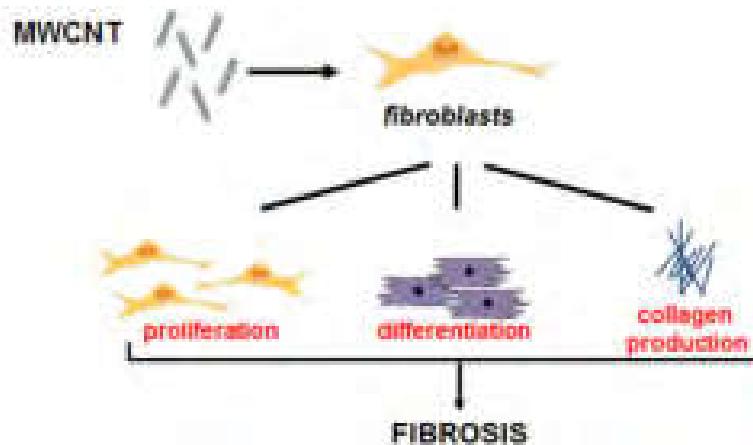
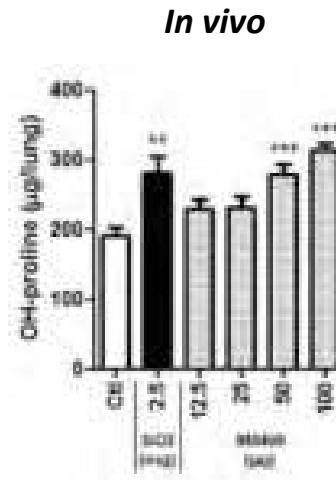


Chortarea et al. ACS Nano 2017

CNTs stimulate the proliferation of fibroblasts *in vitro*



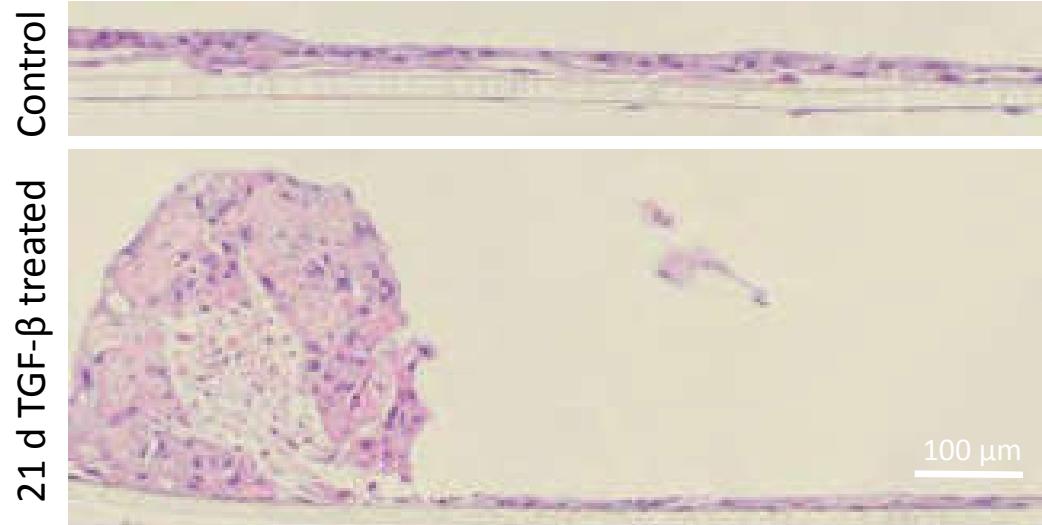
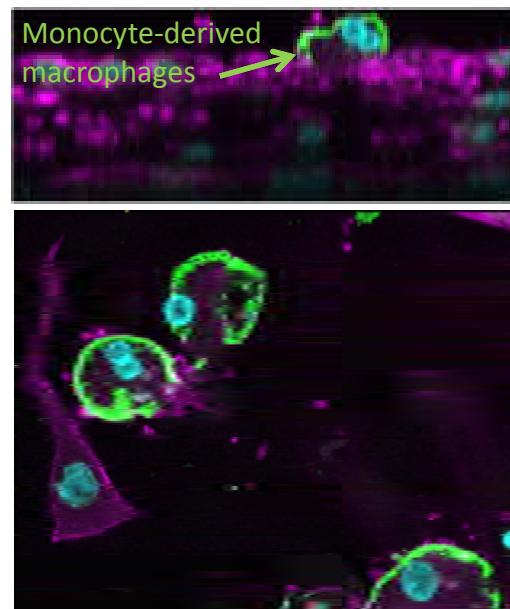
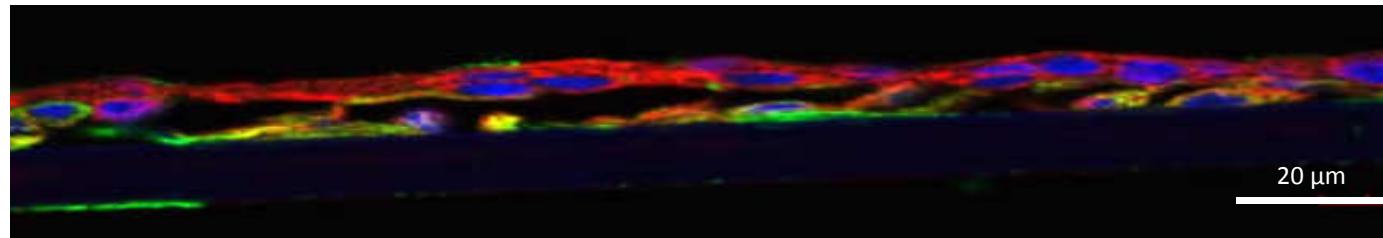
CNTs stimulate the proliferation of fibroblasts *in vitro*



- 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

EpiAlveolar™ lung model to predict fibrosis

- Nuclei
- Epithelial cells
- Fibroblasts
- Endothelial cells

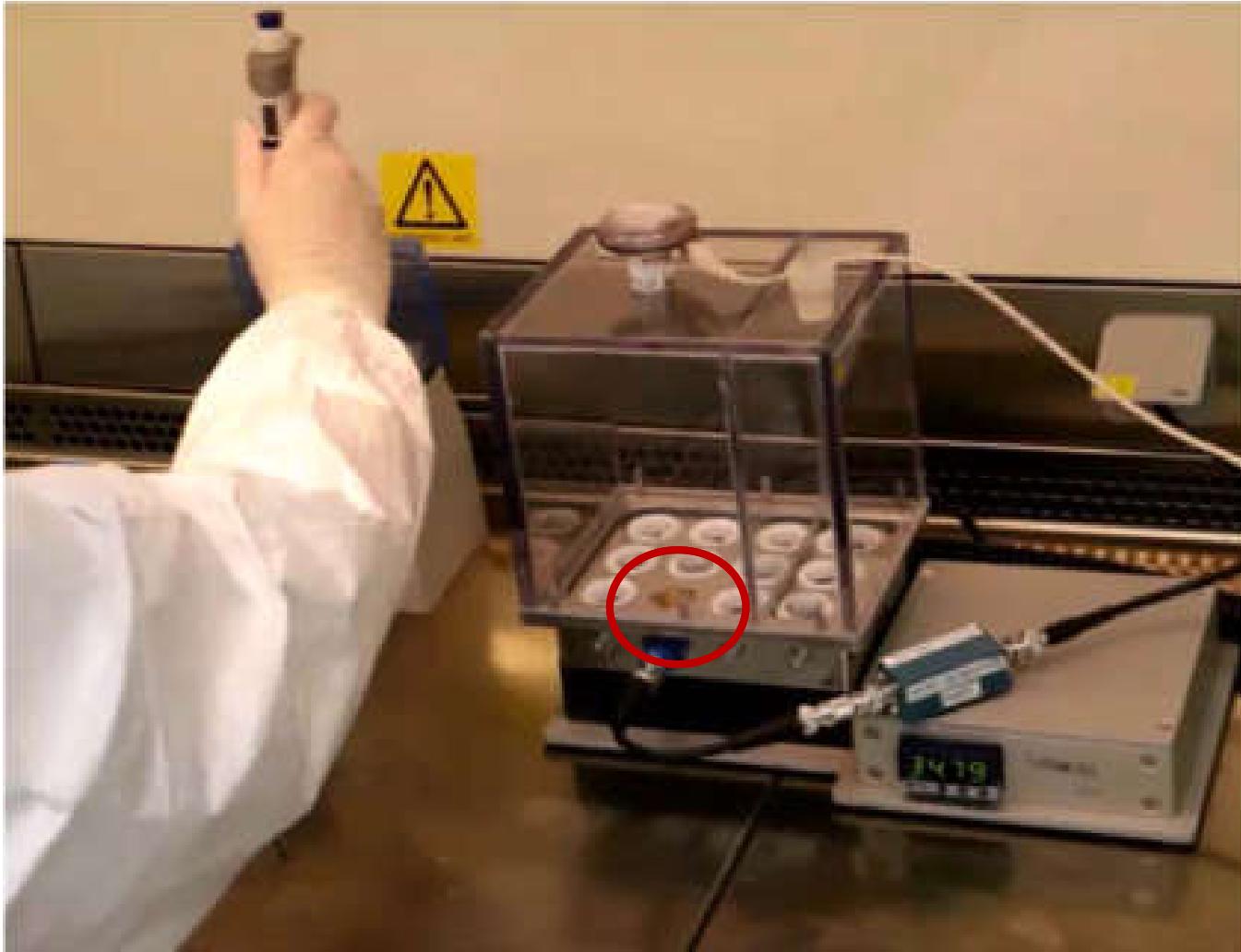


Barosova et al. ACS Nano (2020)

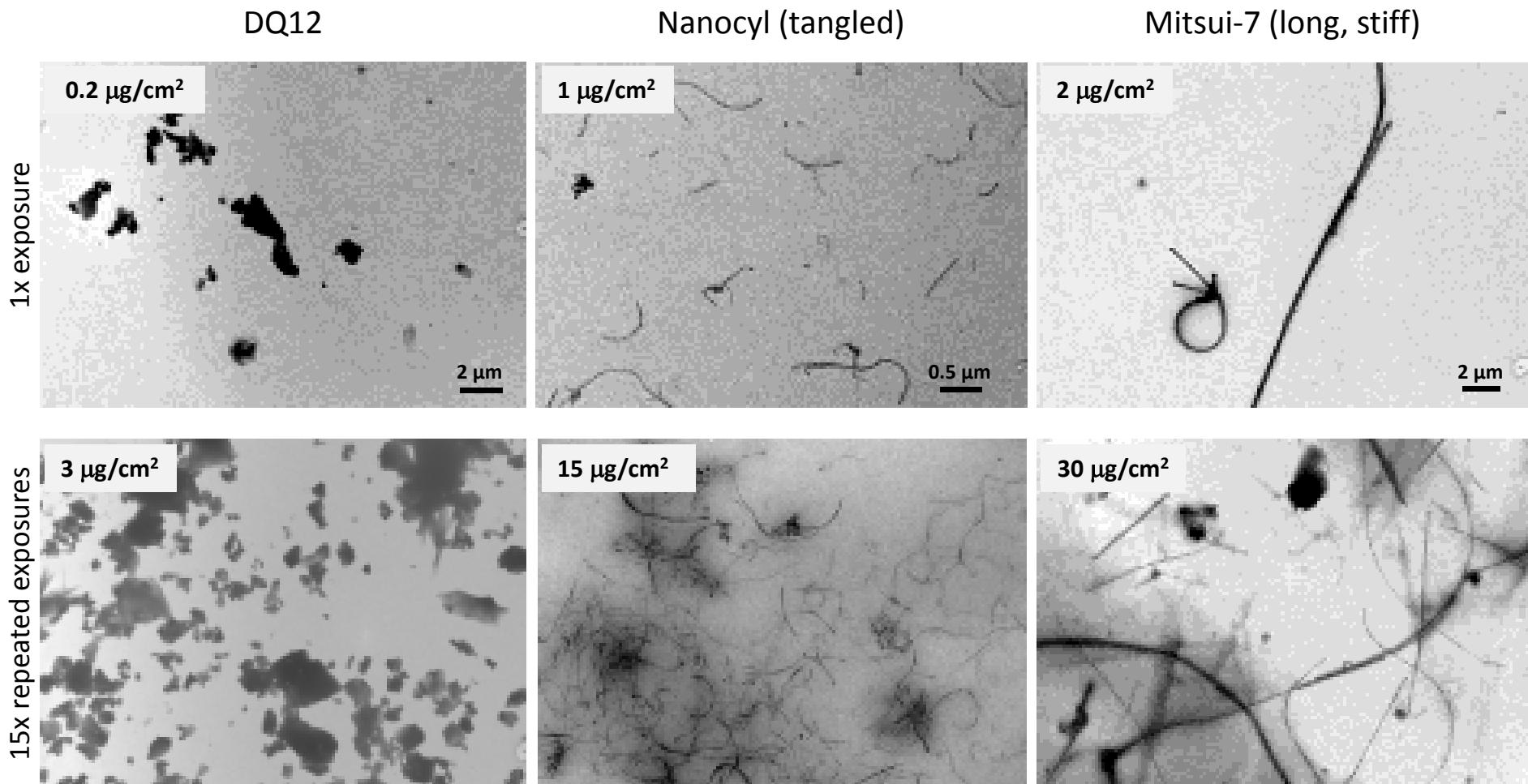


Repeated (nano)material exposures

S. Chortarea, H. Barosova

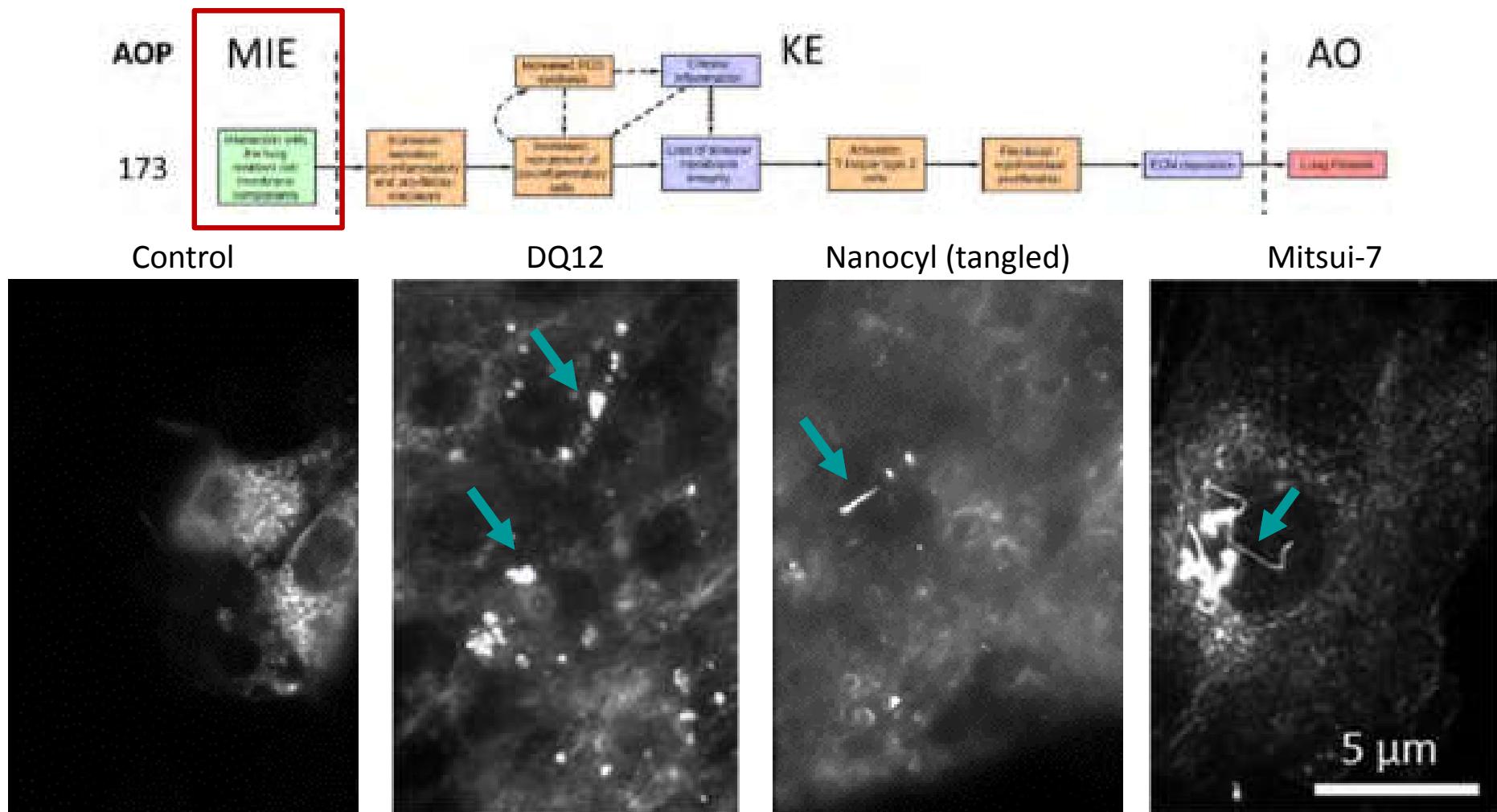


Repeated (nano)material exposures



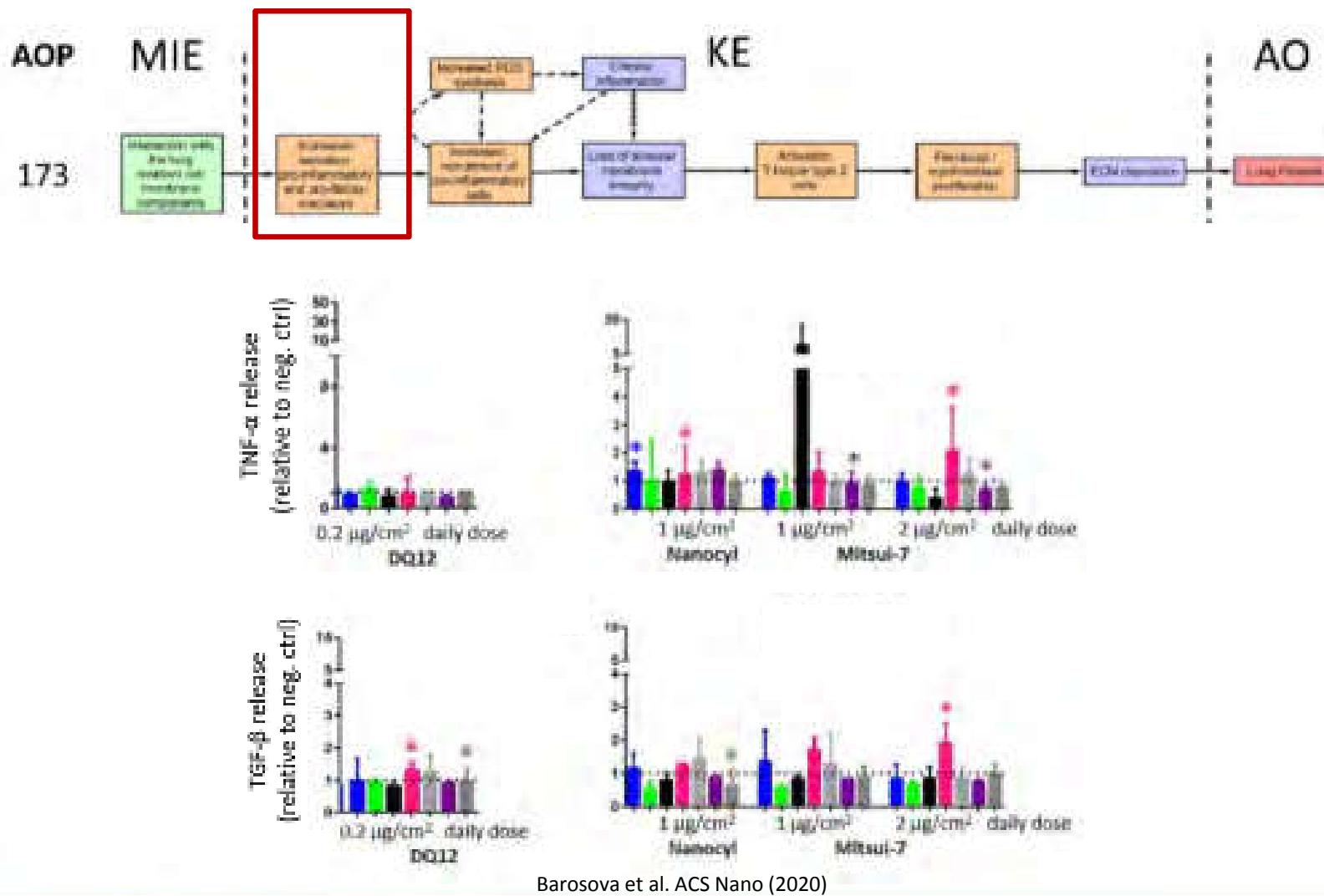
Barossova et al. ACS Nano (2020)

Repeated (nano)material exposures to EpiAlveolarTM cells

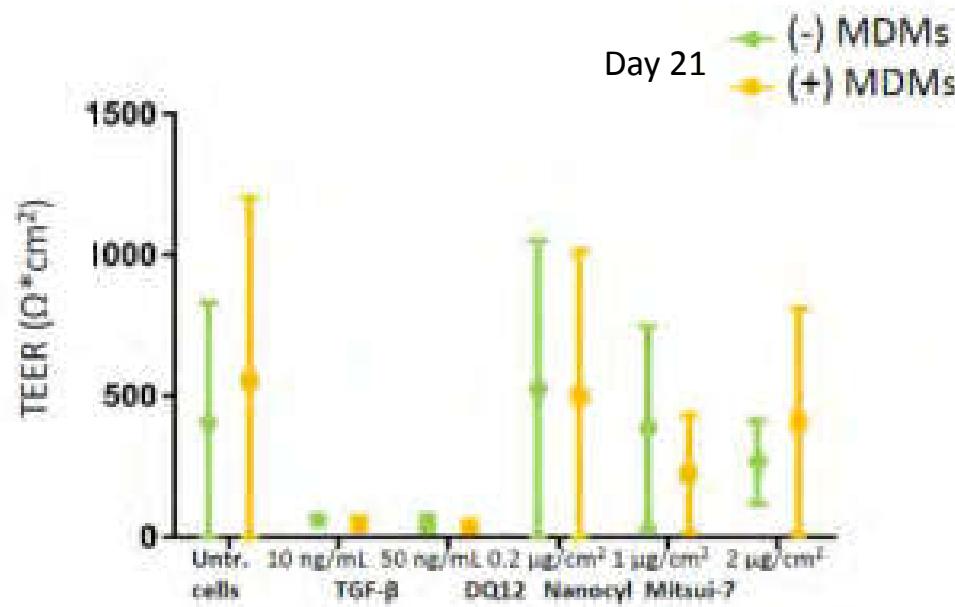
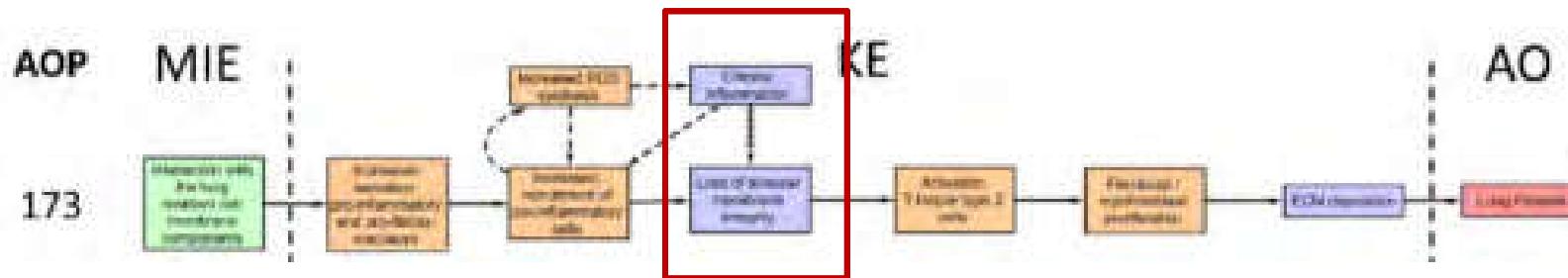


Barosova et al. ACS Nano (2020)

Repeated (nano)material exposures to EpiAlveolar™ cells

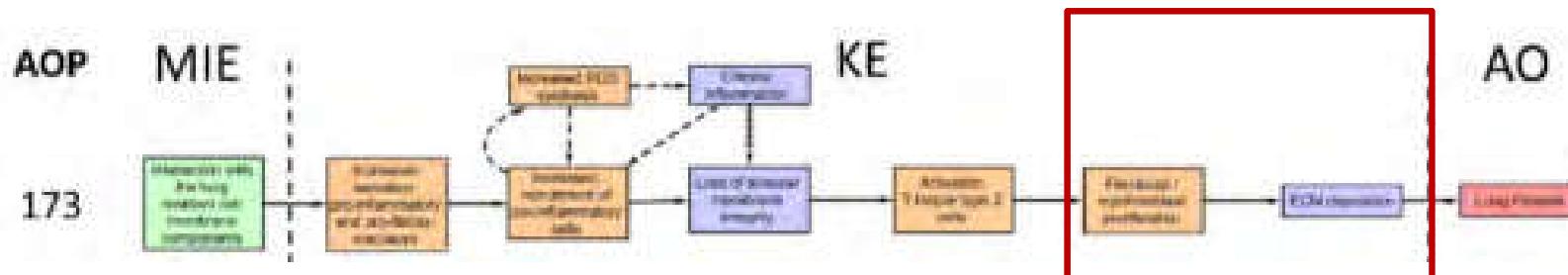


Repeated (nano)material exposures to EpiAlveolar™ cells



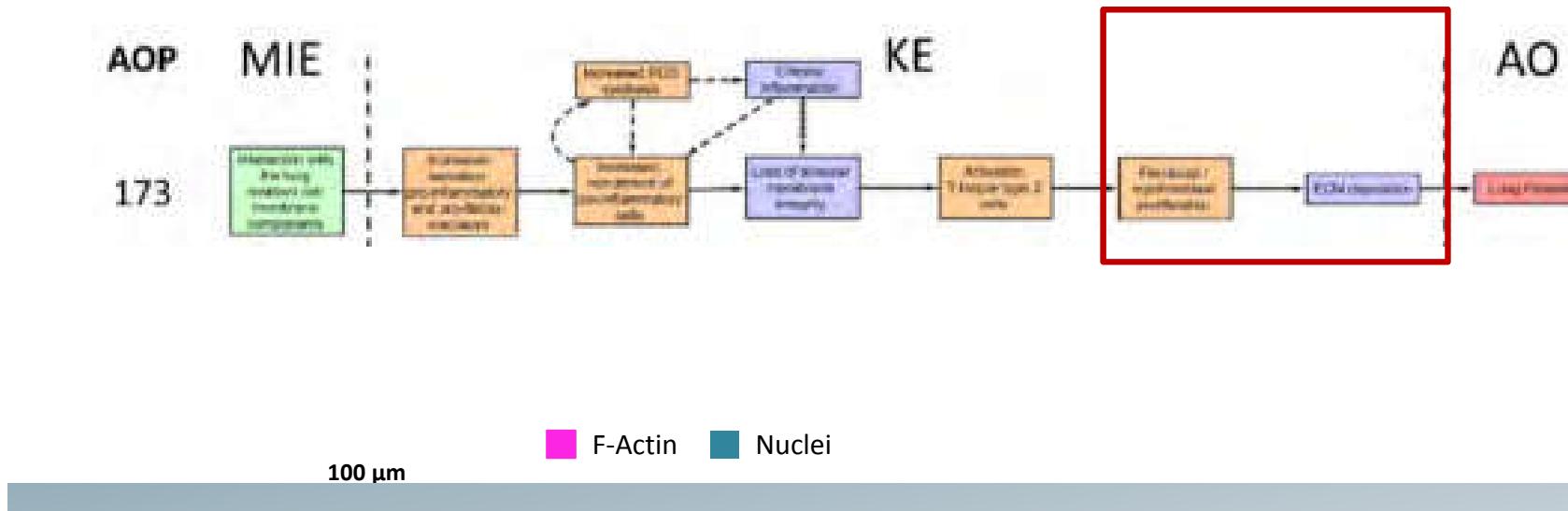
Barosova et al. ACS Nano (2020)

Repeated (nano)material exposures to EpiAlveolarTM cells



Barosova et al. ACS Nano (2020)

Repeated (nano)material exposures to EpiAlveolar™ cells



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



Mitsui-7
~ 30 $\mu\text{g}/\text{cm}^2$

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



Hana Barosova

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



PATROLS
Advanced Tools for NanoSafety Testing



FONDATION EGON NALL
POUR LA RECHERCHE IN VITRO

PETA INTERNATIONAL
SCIENCE CONSORTIUM LTD.
Advancing 21st Century Toxicology

ENI-SNF
Schweizerische Nationalfonds
für Förderung der wissenschaftlichen Forschung

**BIO-INSPIRED
MATERIALS**
NATIONAL CENTER OF COMPETITIVE
RESEARCH



This project has received funding from the European
Union's Horizon 2020 research and innovation
programme under the Marie Skłodowska-Curie grant
agreement No 705602.



THANKS FOR YOUR ATTENTION



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

www.patrols-h2020.eu