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Development of an *In Vitro* System to Assess the Inhalation Toxicity of Nanomaterials

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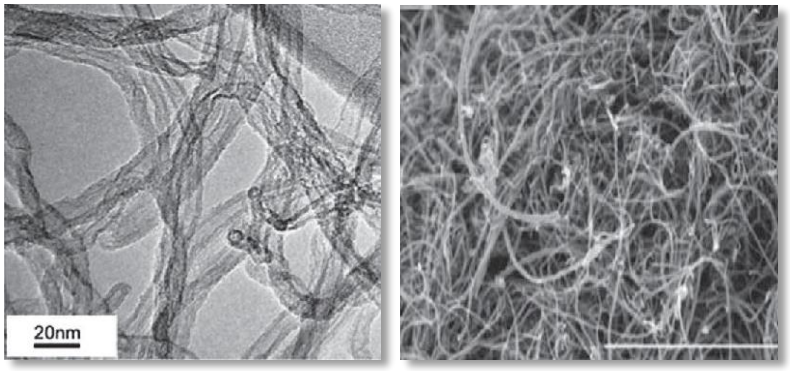
PETA INTERNATIONAL SCIENCE CONSORTIUM LTD.
Advancing 21st Century Toxicology

Introduction – Multi-walled carbon nanotubes (MWCNTs)

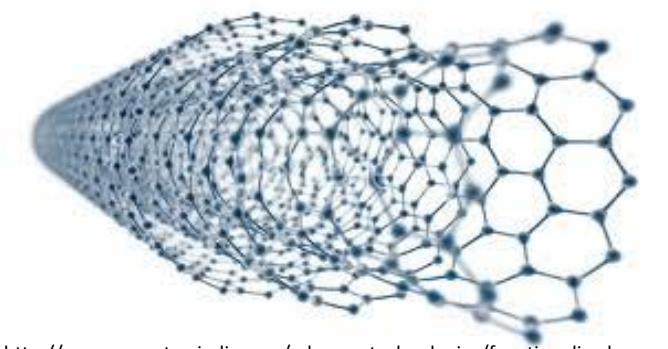
Multi-walled carbon nanotubes (MWCNTs)[1]



CNTs production: **11-1000** tons/year[2]

Potential human exposure:

- occupational
- use of consumer products/disposal

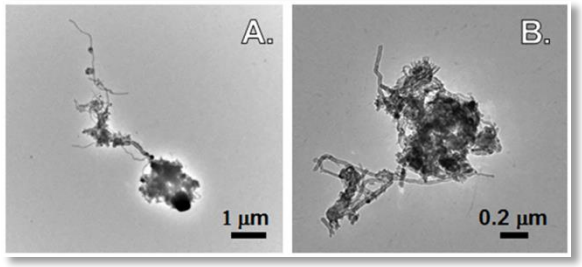


http://www.exportersindia.com/ad-nano-technologies/functionalized-mwcnt-shimoga-india-776051.htm

Examples of commercial applications

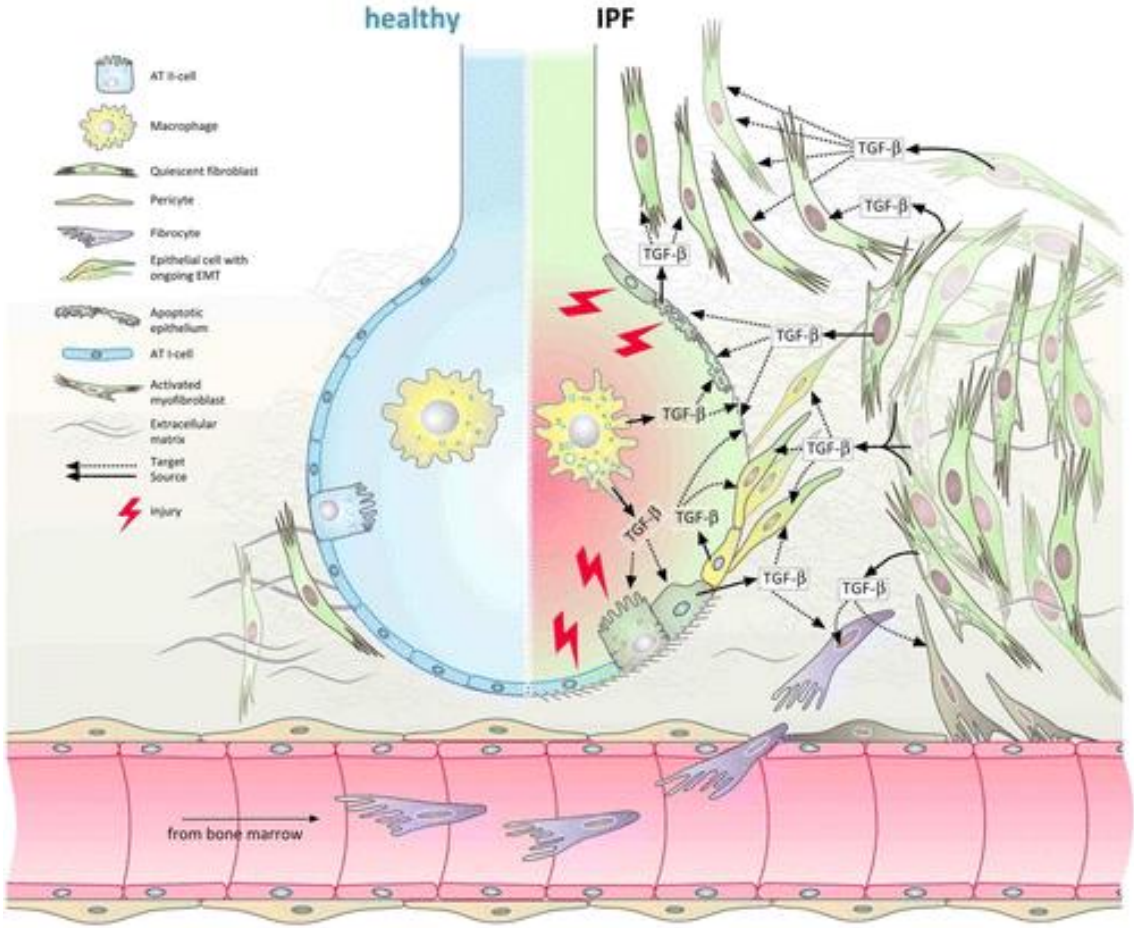
www.bike-eu.com; www.nanotechmag.com; www.cdc.com; www.future-carbon.de

Samples collected in MWCNT facilities[3]



[1] Thurnherr et al. (2009), Nanotoxicology. [2] Piccinno et al.,(2012); J Nanopart Res. [3] Shvedova et al. (2016), Plos One.

Introduction – Pulmonary fibrosis



Hypothetical scheme of the role of **TGF-β** in idiopathic pulmonary fibrosis (IPF) pathogenesis and potential sources of the activated myofibroblast[1].

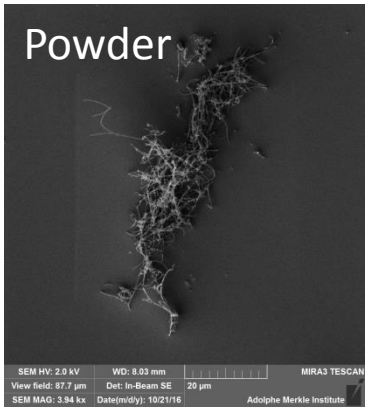
=> Mono- and co-culture **lung cell models** for the prediction of **(pro-) fibrotic** events upon exposure to CNTs.

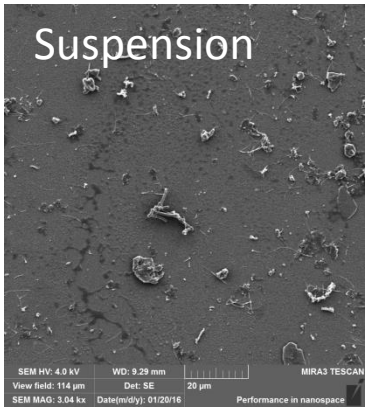
[1]Fernandez I.E., Eickelberg O. (2009), Proceedings of The American Thoraic Society.

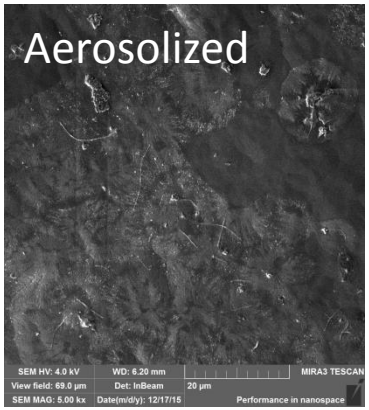
Carbon nanotubes exposures

Vitrocell® Cloud system:
NM Generation and
Exposure System

Type of CNTs	Length (µm)	Width (nm)	Impurities	Total deposition in ALI per exposure
Mitsui-7 [1]	13	40 – 50	< 1 %	1 µg/cm^2
Nanocyl 7000[2]	1,5	9,5	< 1 %	0,5 µg/cm^2







Mitsui-7 MWCNTs

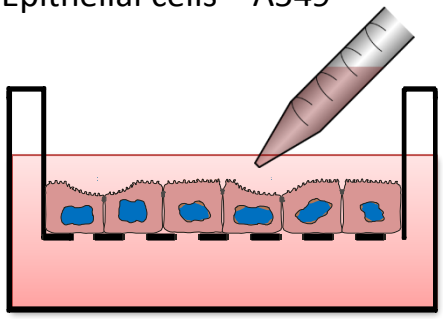
[1] Poland *et al.* (2008); *Nat. Nanotech.* [2] Technical Data Sheet: NC7000™, 01/2016 online: http://www.nanocyl.com/wp-content/uploads/2016/02/Technical-Data-Sheet-NC7000-V07.pdf

Barosova et al., manuscript in preparation

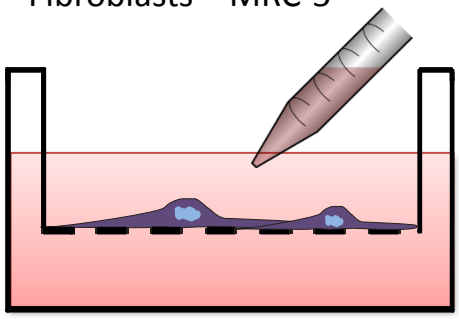
Cell models - Monocultures

Mitsui-7 : 5, 10, 20µg/mL dispersed in complete medium

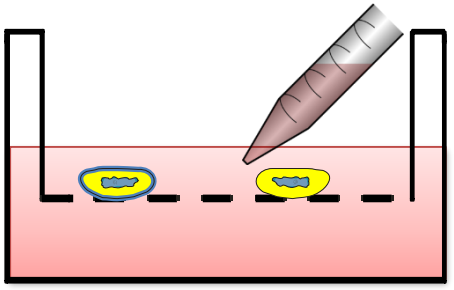
Epithelial cells – A549



Fibroblasts – MRC-5

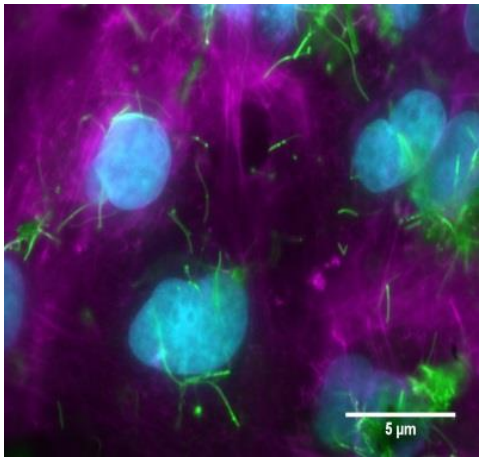


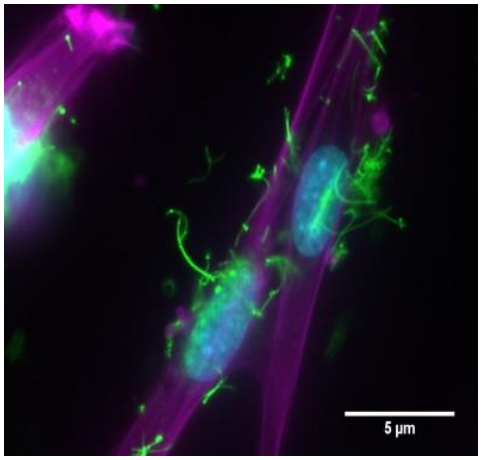
Macrophages – THP-1

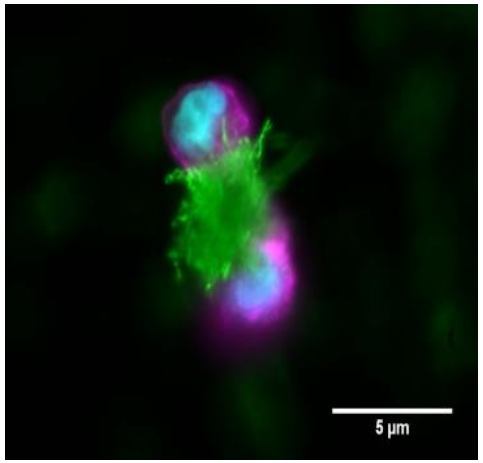


10 µg/ml Mitsui-7, 24h post-exposure

Dark field-fluorescence imaging







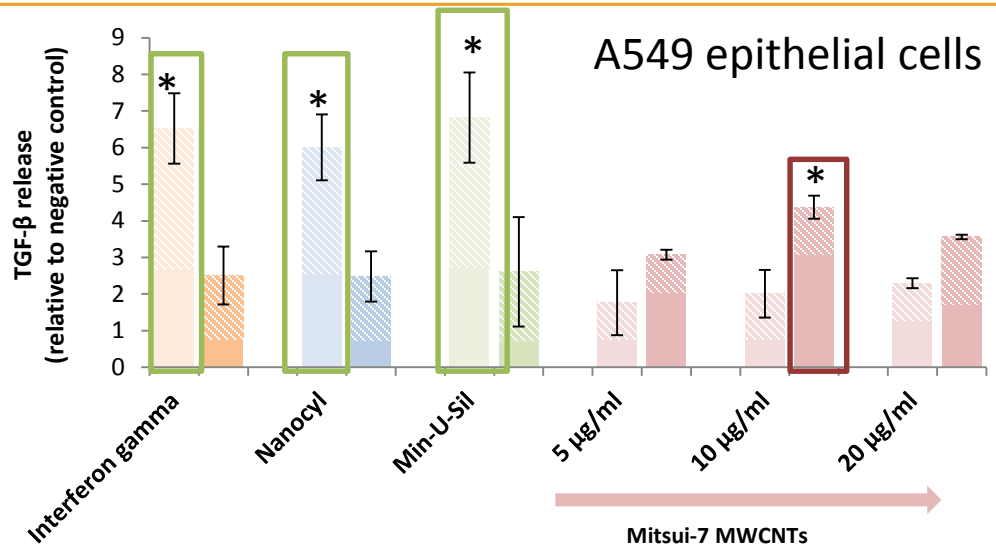
F-actin Cell nuclei Mitsui-7

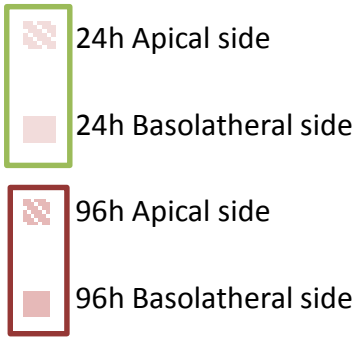
Chortarea et al., manuscript in preparation

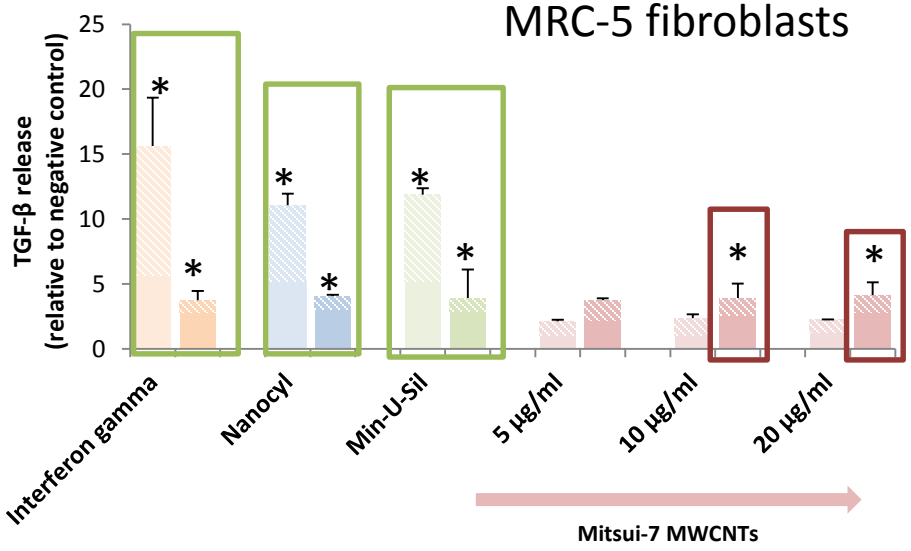
Exposure scenarios + endpoints

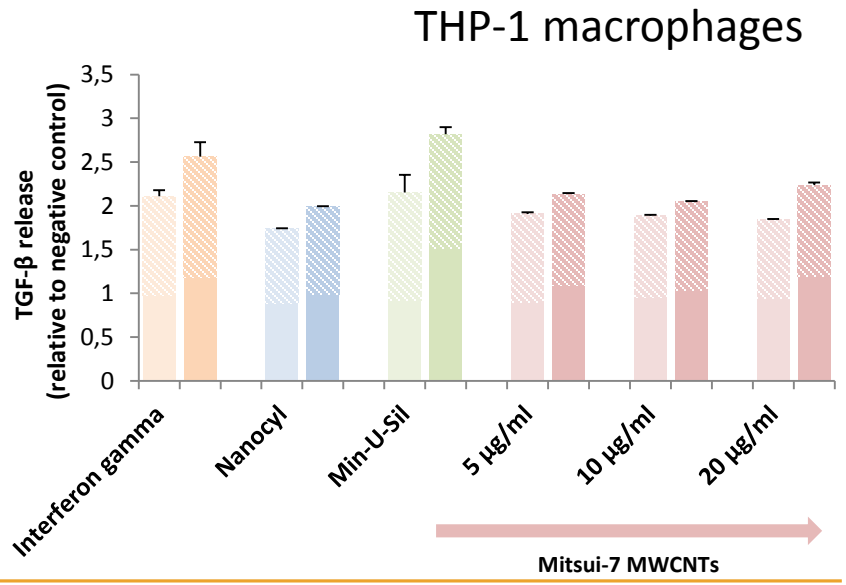
	Cell model	Exposure method	Time-points for sample collection	Investigated endpoints
Monocultures	A549	suspension	24h, 96h	Cytotoxicity (LDH) Oxidative stress (GSH) Pro-fibrotic response (TGF-β, OPN, PDGF-AA) Pro-inflammation (IL-8, TNF-α, IL-1β) Cell morphology (LSM) Cell proliferation (BrdU)
	MRC-5	suspension	24h, 96h	
	THP-1	suspension	24h, 96h	

Results – Pro-fibrotic response in mono-cultures







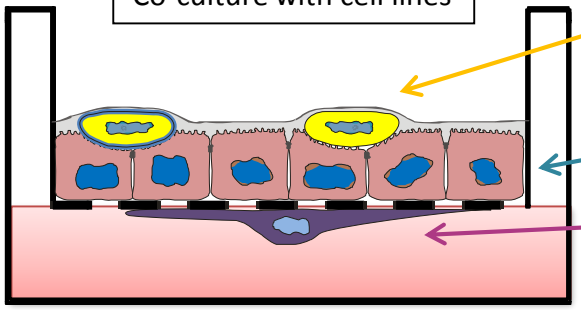


n=3; [Iγ]=1µg/ml; Error bars: SEM; * statistically significant increase

Chortarea et al., manuscript in preparation

Cell models – Co-culture models

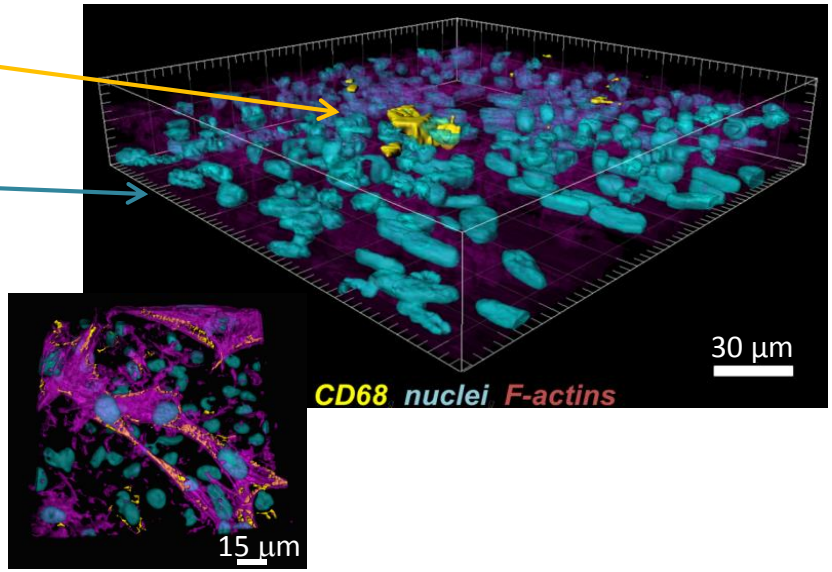
Co-culture with cell lines



Macrophages (THP-1)

Epithelial cells (A549)

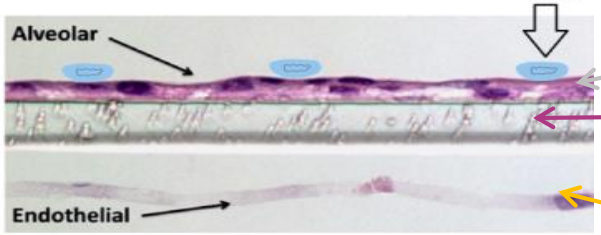
Fibroblasts (MRC-5)



MatTek CORPORATION

EpiAlveolar™ model (primary cells)

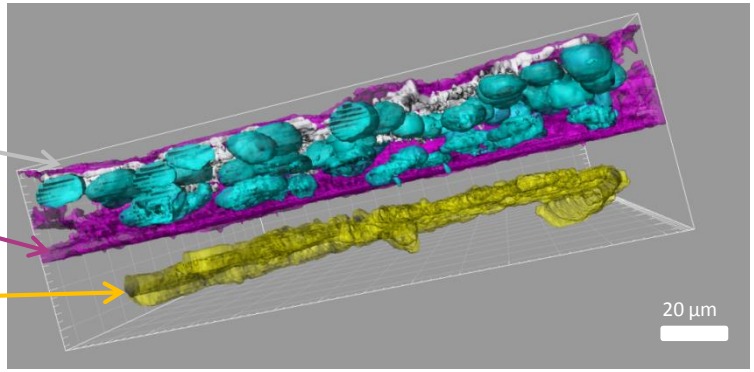
Macrophages (THP-1)



Primary alveolar epithelial cells

Fibroblasts

Primary endothelial cells



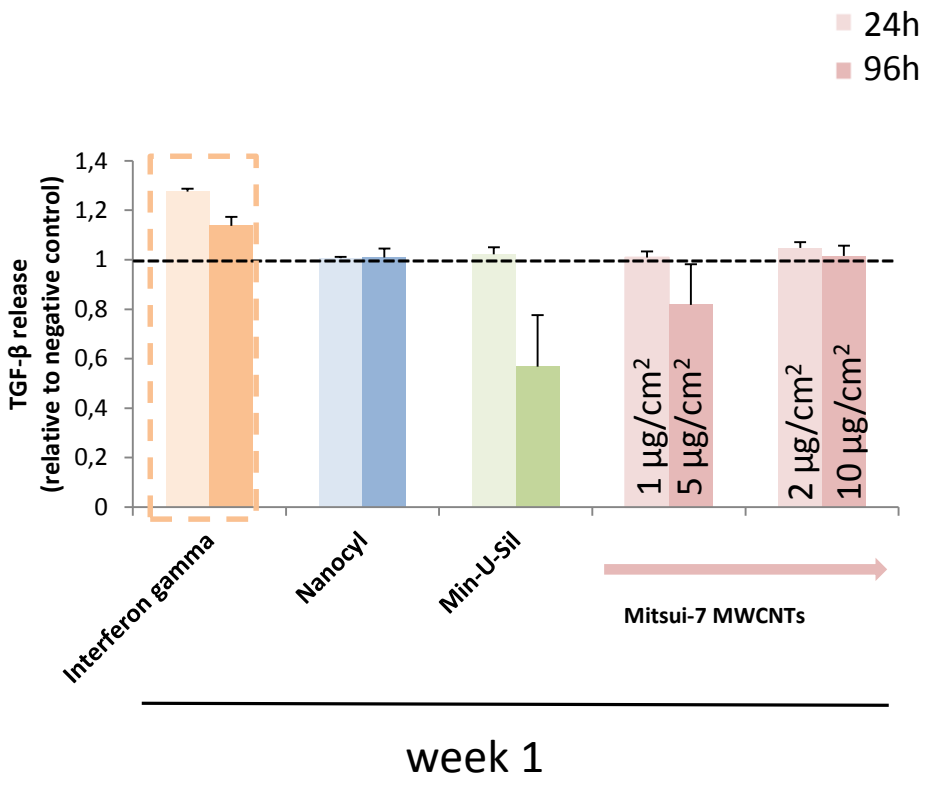
Barosova et al., manuscript in preparation

Exposure scenarios + endpoints

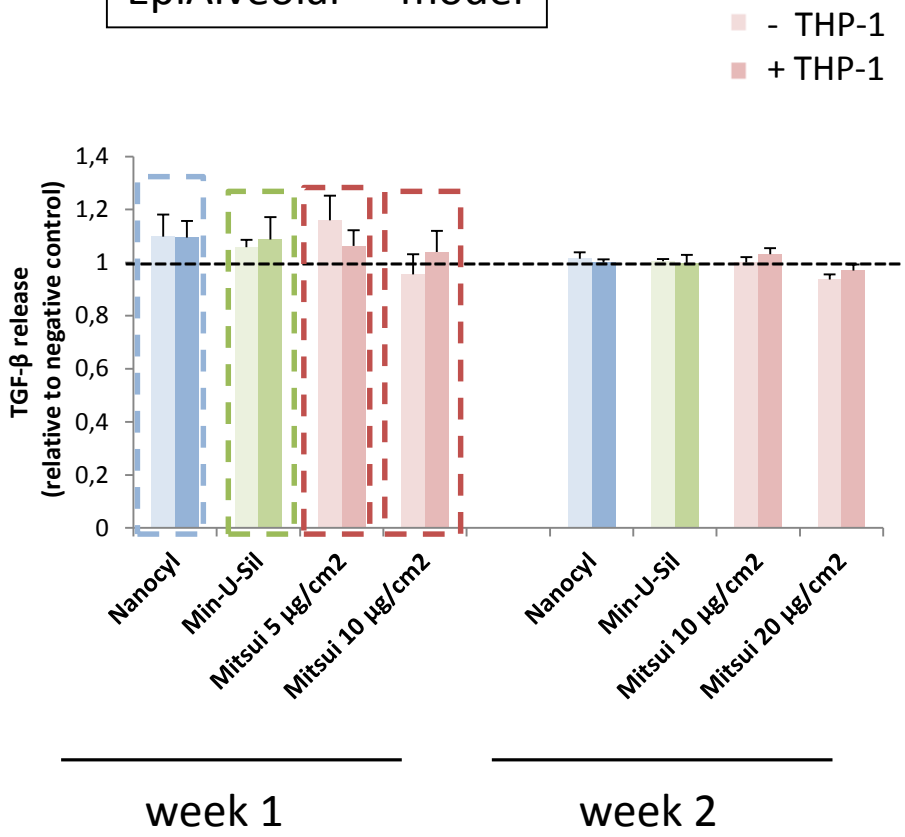
	Cell model	Exposure method	Time-points for sample collection	Investigated endpoints
Co-culture models	Cell-lines co-culture	Air-liquid interface (VITROCELL© Cloud system)	24h, 96h	Cytotoxicity (LDH) Oxidative stress (GSH) Pro-fibrotic response (TGF-β, OPN, PDGF-AA) Pro-inflammation (IL-8, TNF-α, IL-1β) Cell morphology (LSM) Cell proliferation (BrdU)
	EpiAlveolar™ model (primary cells)	Air-liquid interface (VITROCELL© Cloud system)	1 week (96h), 2 weeks	

Results – Pro-fibrotic response in co-cultures

Co-culture with cell lines



EpiAlveolar™ model



n=3; [Iγ]=1µg/ml; Error bars: SEM

Barosova et al., manuscript in preparation

Conclusions

- The cells were exposed to 5 – 20 µg/ml (suspension), or 1 – 20 µg/cm^2 (ALI). VITROCELL Cloud system exposures resulted in homogenous repeatable dose-dependent MWCNT deposition.

	Cell model	Increase 24 h post-exposure	Increase 96 h post-exposure	2 weeks post-exposure
Monocultures	A549	-	OPN, PDGF-AA and TGF-β	N/A
	MRC-5	IL-1β and OPN	TGF-β	N/A
	THP-1	IL-1β	-	N/A
Co-culture models	Cell-lines co-culture	IL-1β, TNF-α, TGF-β, PDGF-AA and OPN	IL-1β and TNF-α	N/A
	EpiAlveolar™ model	N/A	TNF-α, TGF-β, OPN	TNF-α

- Monocultures and suspension exposures (high concentrations) show significant increase in pro-fibrotic markers.
- No significant increase but only trends for both co-culture models for all cytokines was observed.

Outlook

- The concentration of 10 µg/cm^2 of Mitsui (obtained after 5 days of exposures) corresponds to lowest concentration used *in vivo* (Snyder-Talkington et al., 2013, Toxicology and Applied Pharmacology, Kobayashi et al., 2010, Toxicology).
- From monoculture experiments it is obvious that pro-longed exposure time is needed to induce a pro-fibrotic response, however, the deposited CNT concentration is not known.
- The air-liquid approach is highly recommended for future experiments.
- To predict the pro-fibrotic potential of CNTs in the co-cultures, the extension of post-exposure time will be tested.

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Thank you for your attention!

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