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The toxicology and functional impact of ultrafine particles on the respiratory mucosa evaluated in a primary cell based air-liquid interface model

Totta Ehret Kasemo[1], Mathilde Delaval[2], Anusmita Das[2,3], Elias Zimmermann[2,3], Jana Panzke[2], Anja Huber[2], Johannes Becker[2,3], Svenja Offer[2], Evelyn Kuhn[2], Seongho Jeong[2,3], Maria Steinke[1,4], Helena Moratin[1], Martin Sklorz[2,3], Ralf Zimmermann[2], Agmal Scherzad[1], Stephan Hackenberg[1], Sebastiano Di Bucchianico[2,3], **Till J. Meyer**[1]

1. **University Hospital Würzburg**, Department of Otorhinolaryngology, Plastic, Aesthetic and Reconstructive Head and Neck Surgery, Wuerzburg, Germany
2. **Helmholtz Zentrum München**, Joint Mass Spectrometry Center (JMSC) at Comprehensive Molecular Analytics (CMA), Neuherberg, Germany
3. **University of Rostock**, Joint Mass Spectrometry Center (JMSC) at Analytical Chemistry, Institute of Chemistry, Rostock, Germany
4. **Fraunhofer Institute for Silicate Research ISC**, Wurzburg, Germany

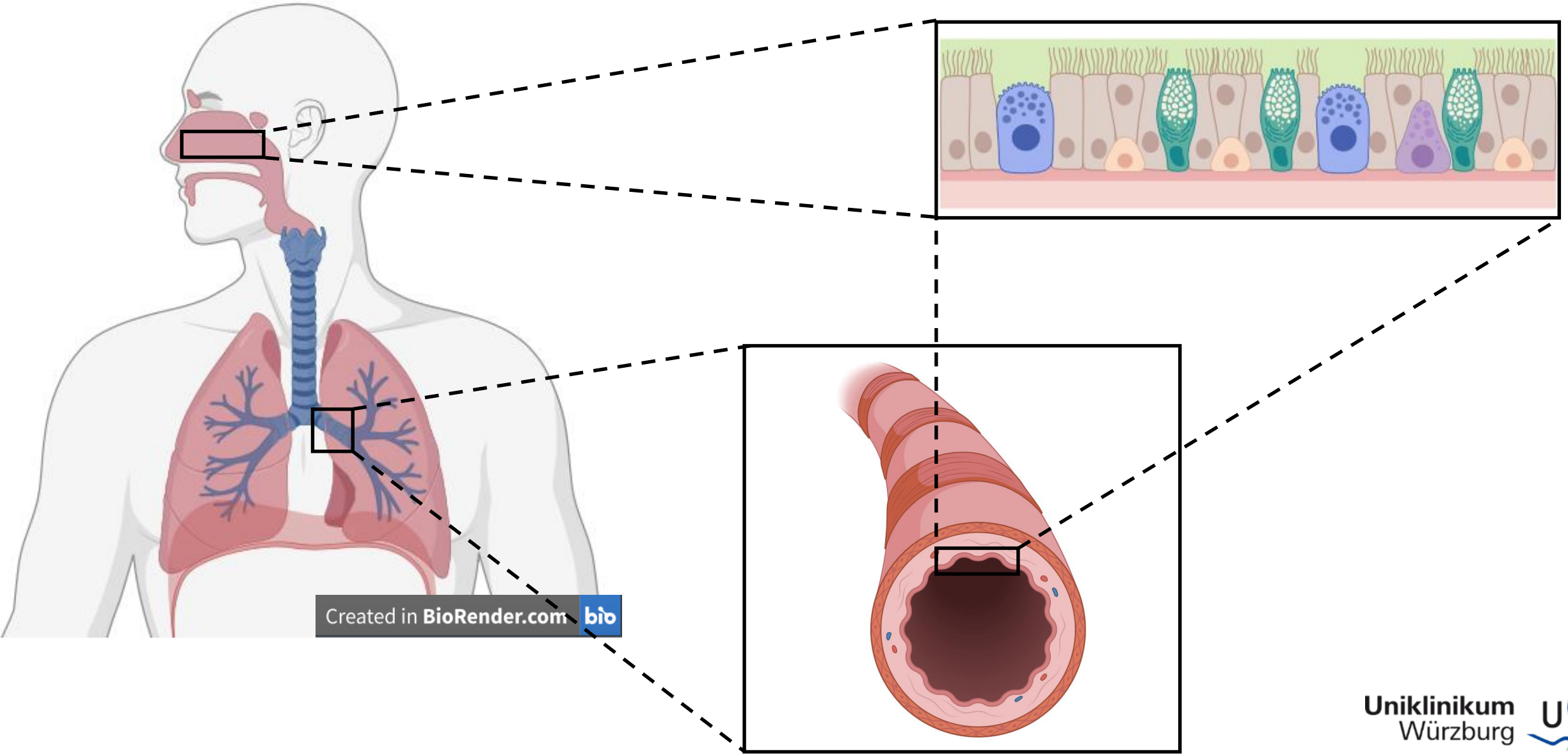
Klinik und Poliklinik für Hals-, Nasen- und Ohrenkrankheiten, plastische und ästhetische Operationen
Direktor: Prof. Dr. S. Hackenberg

HNO

Respiratory mucosa

Picture source: https://ufp.efca.net/

Respiratory mucosa



How to get the best respiratory mucosa model?

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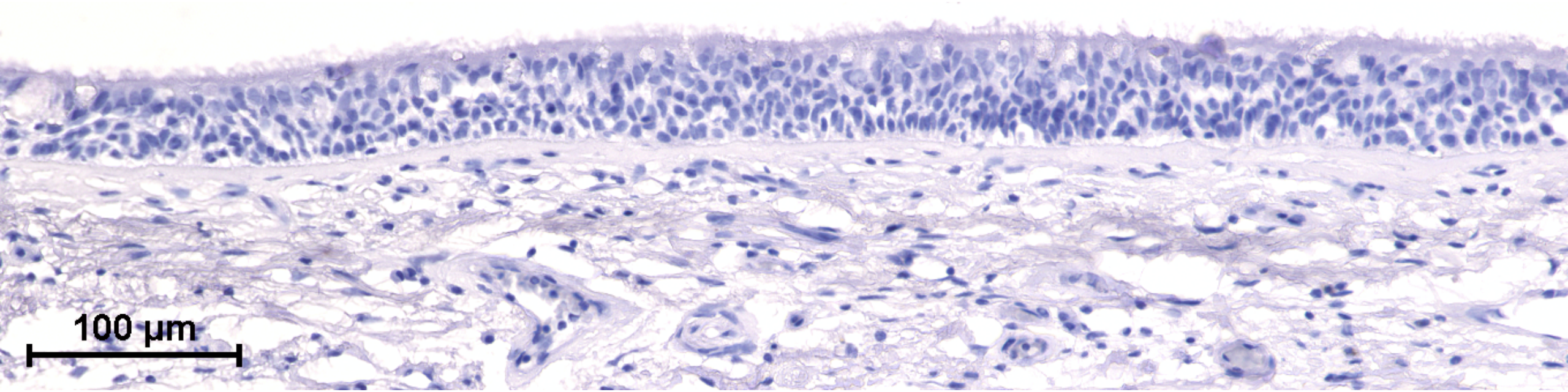

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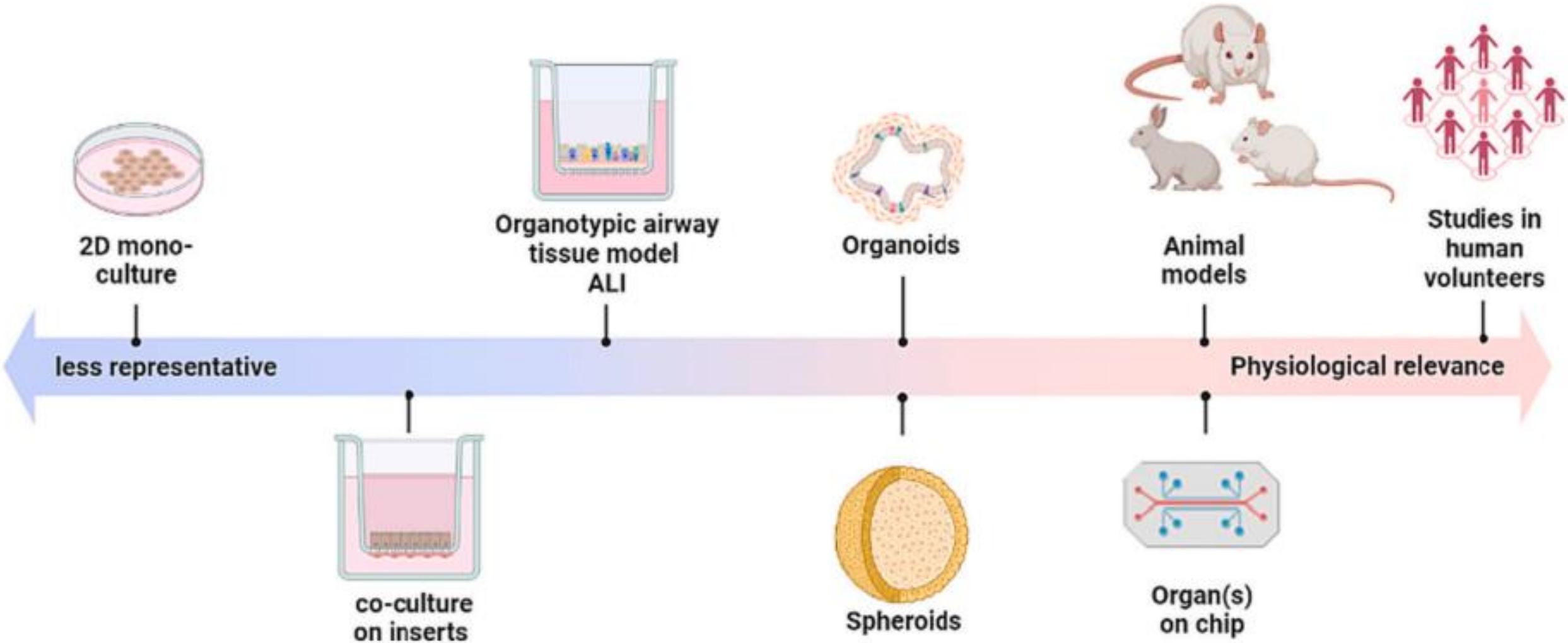
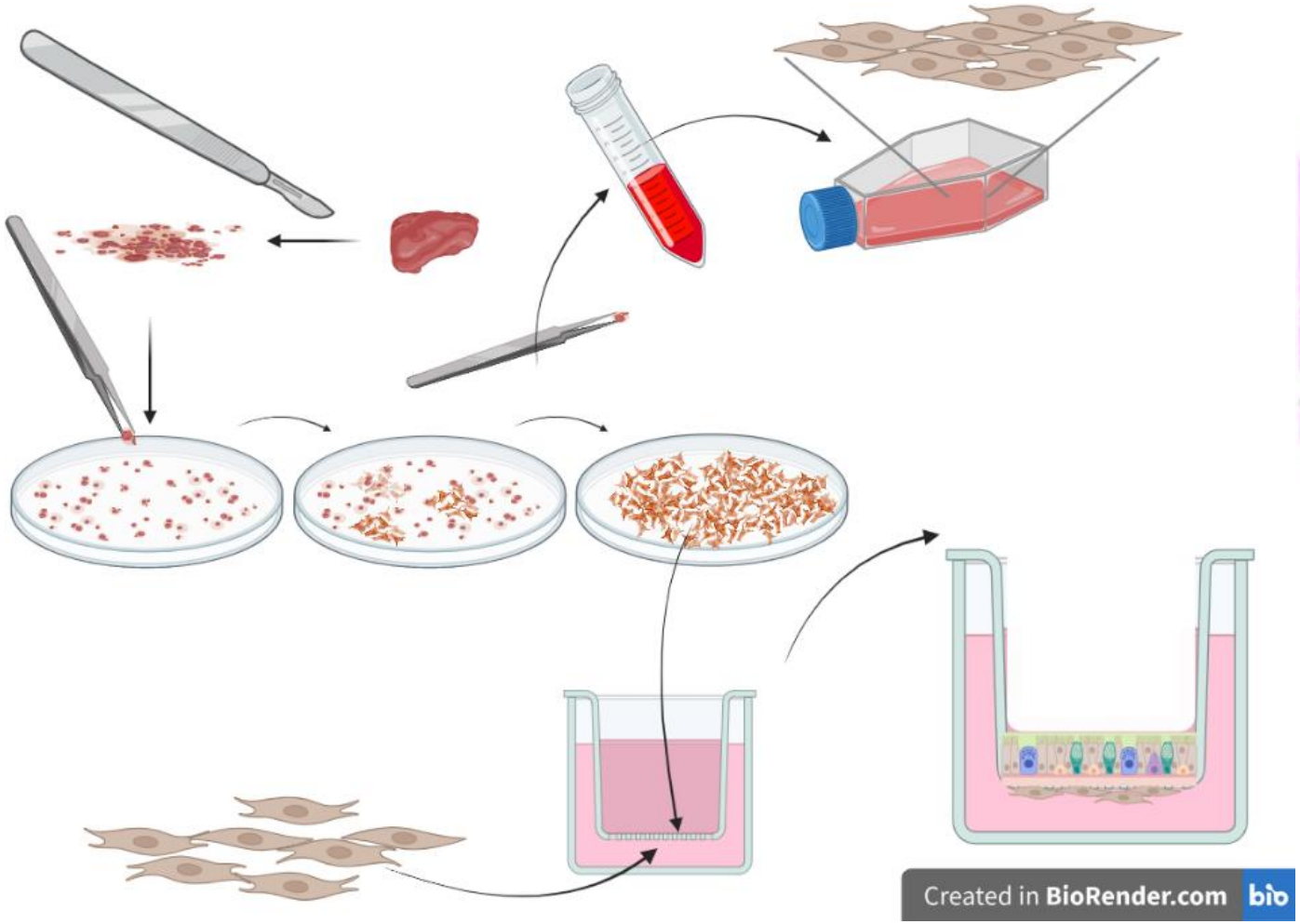
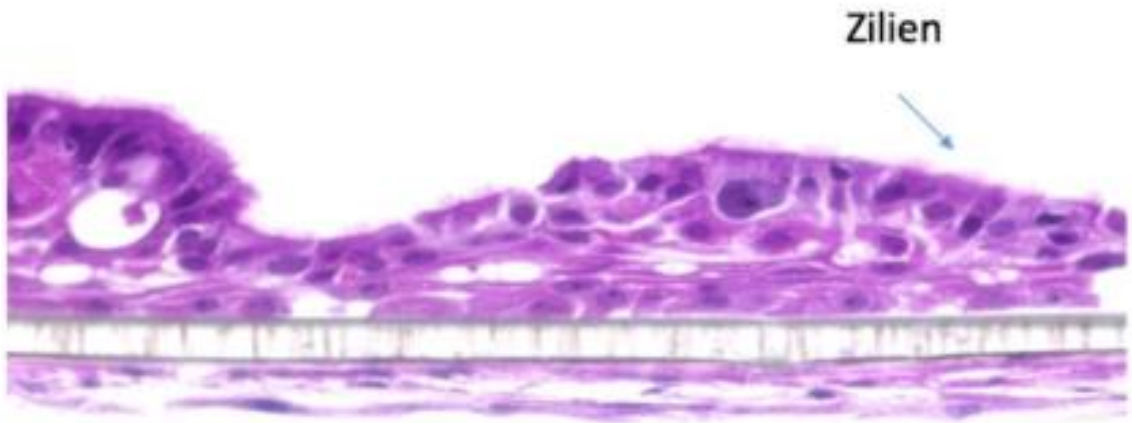


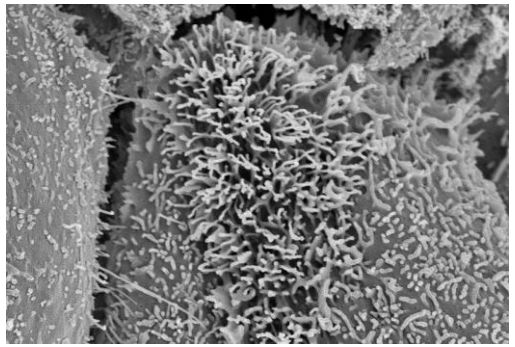
Jaber, et. al.; 2024

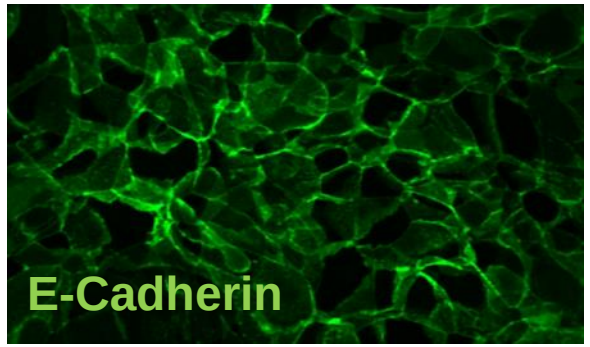
Primary cell based respiratory mucosa model





Meyer, et. al.; 2023





Ultrastructure of the models by TEM

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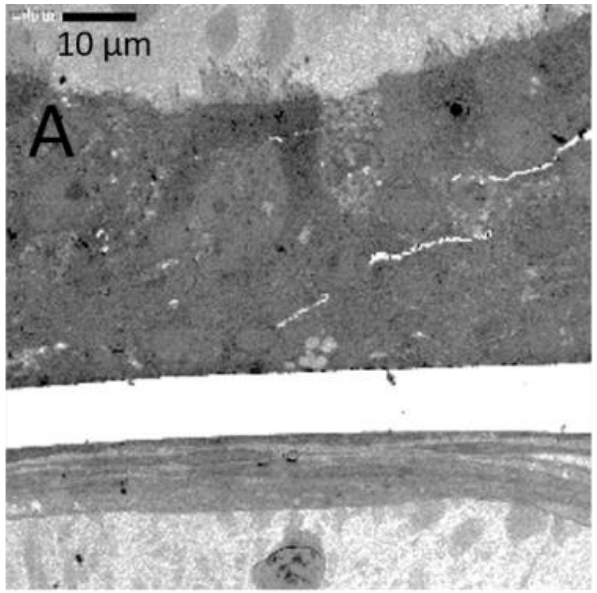
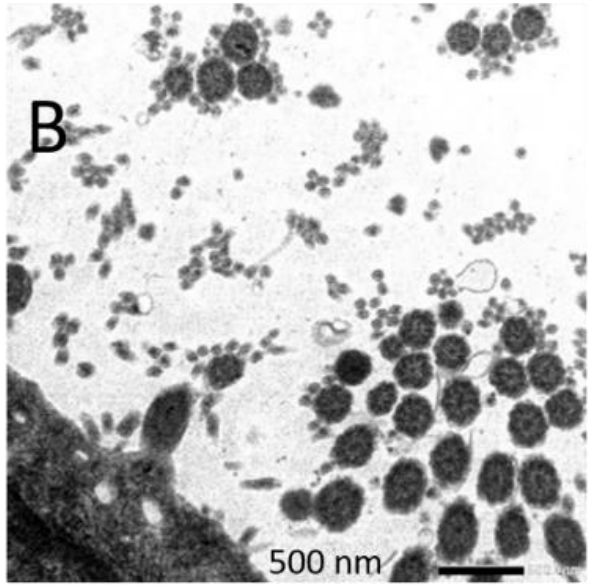
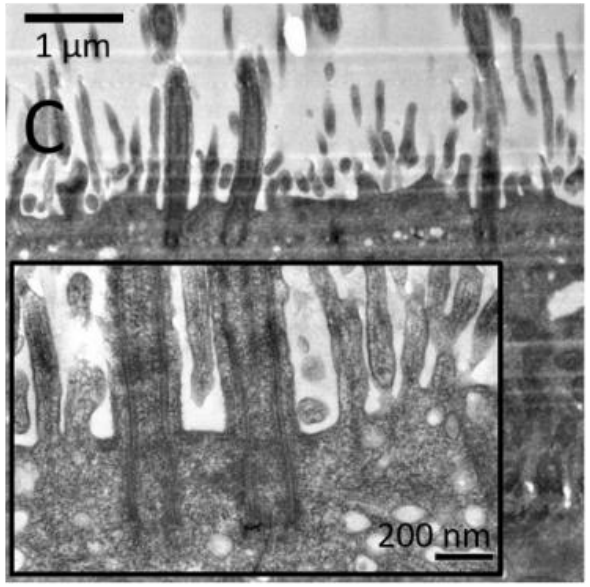


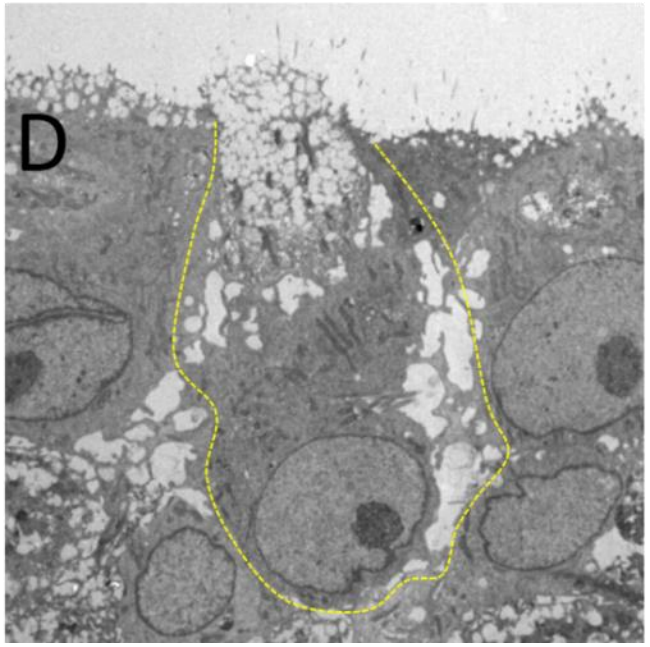
Apical cell culture based on epithelial cells isolated from nasal mucosa biopsies and cultured for a minimum of 26 days at ALI

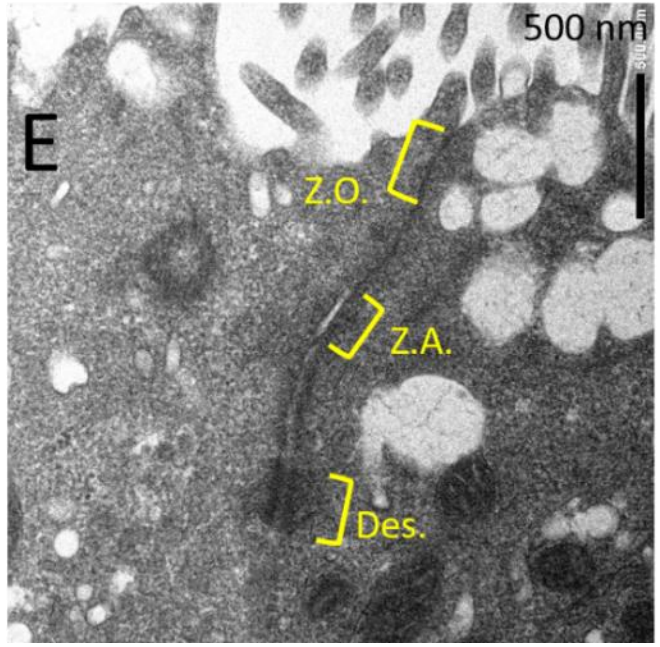
PET membrane

Autolog fibroblasts









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Kinocilia beat – models with function

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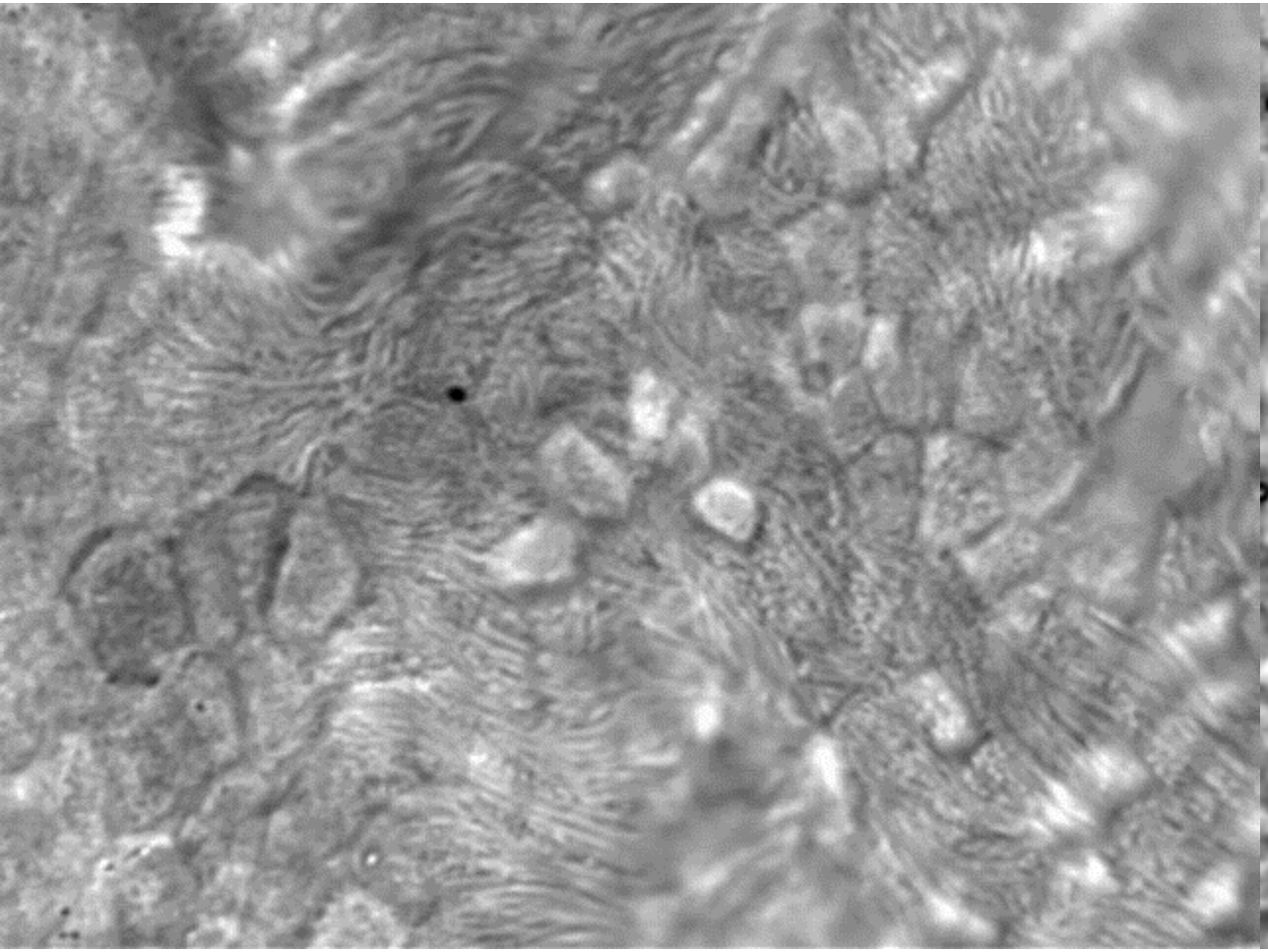
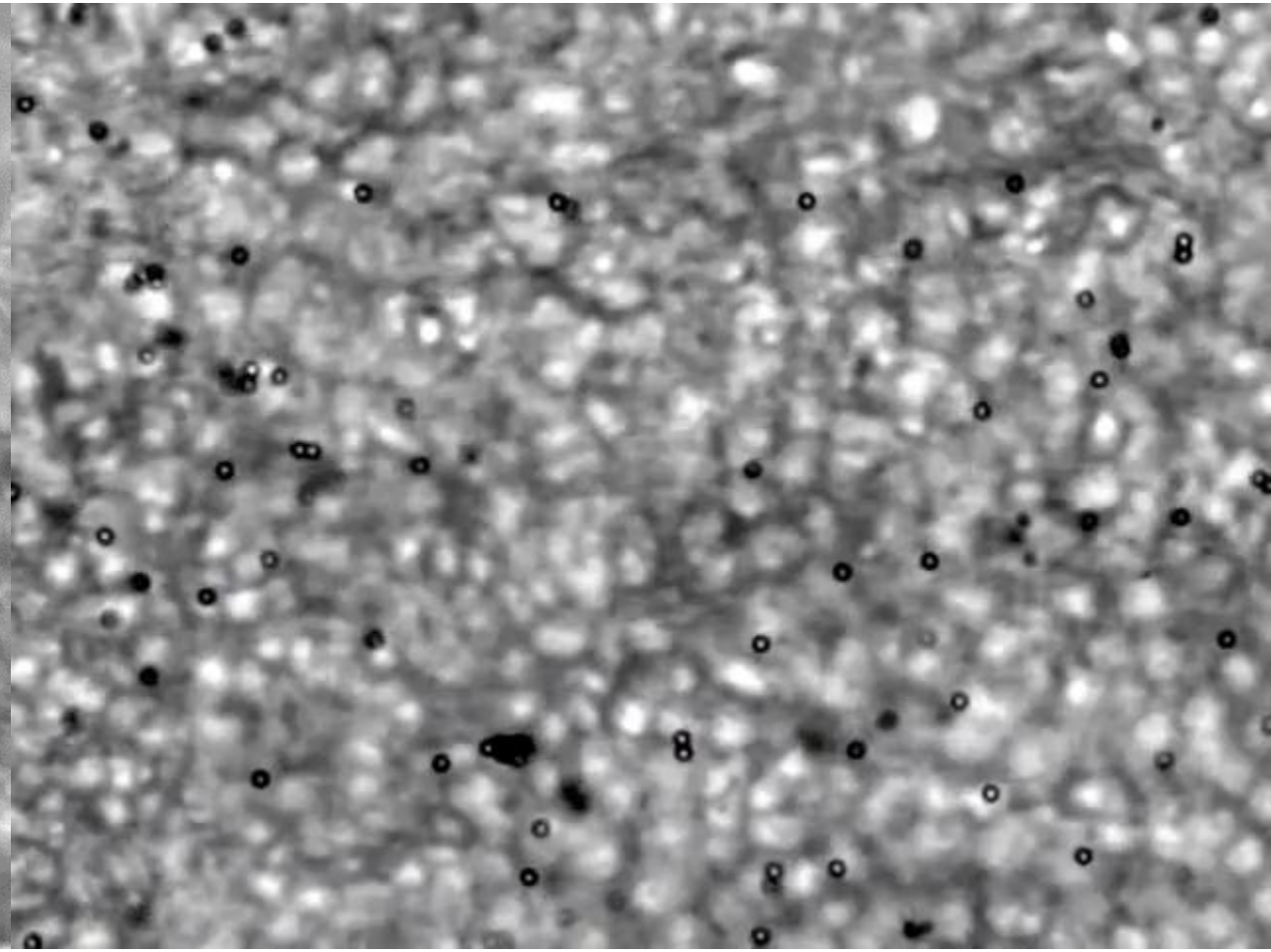
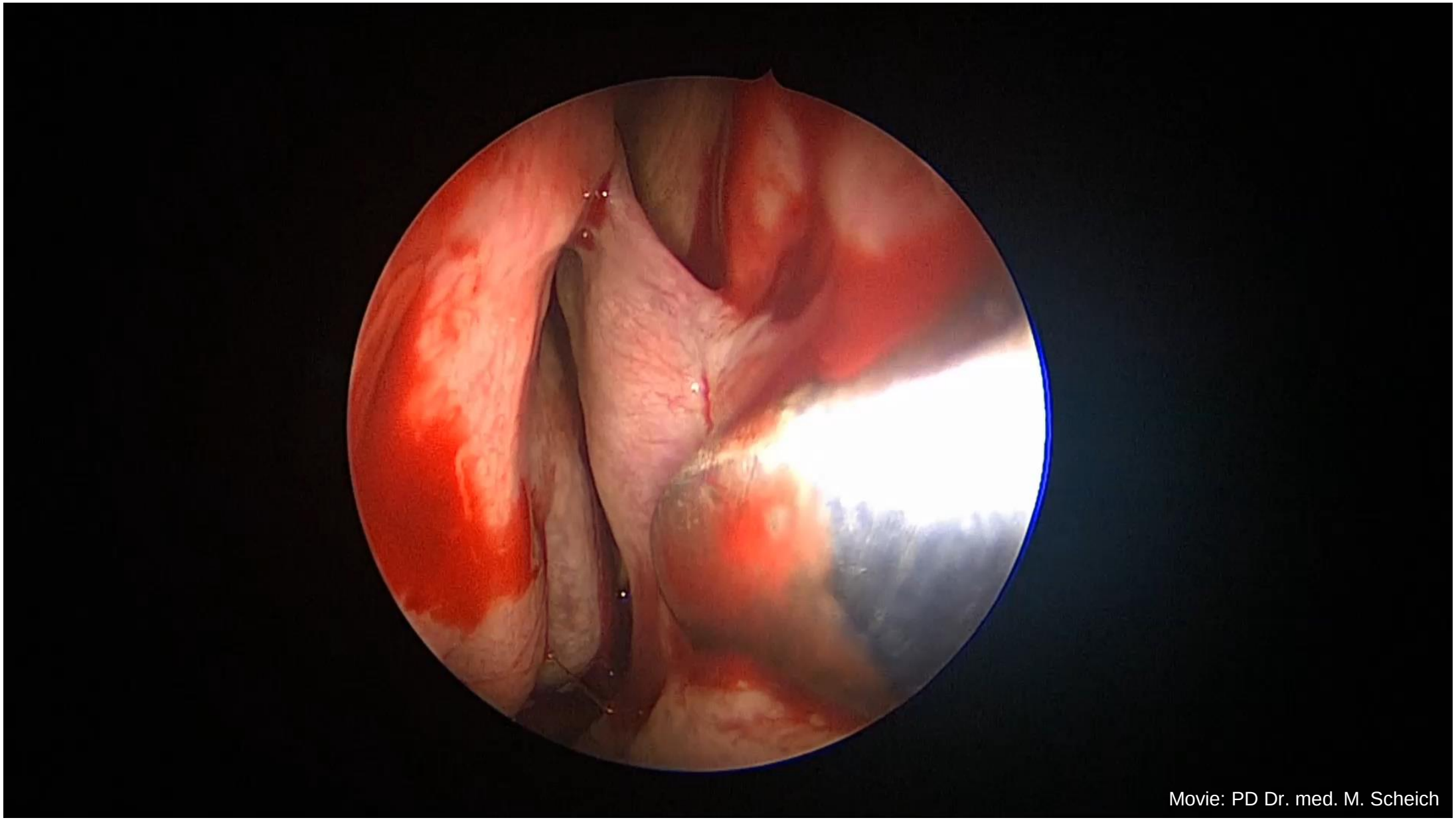




Beads: d = ~3µm

Movie: Dr. Maria Steinke

Source of donor tissue?



Movie: PD Dr. med. M. Scheich

Use of primary cells – an advantage?

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Disadvantages

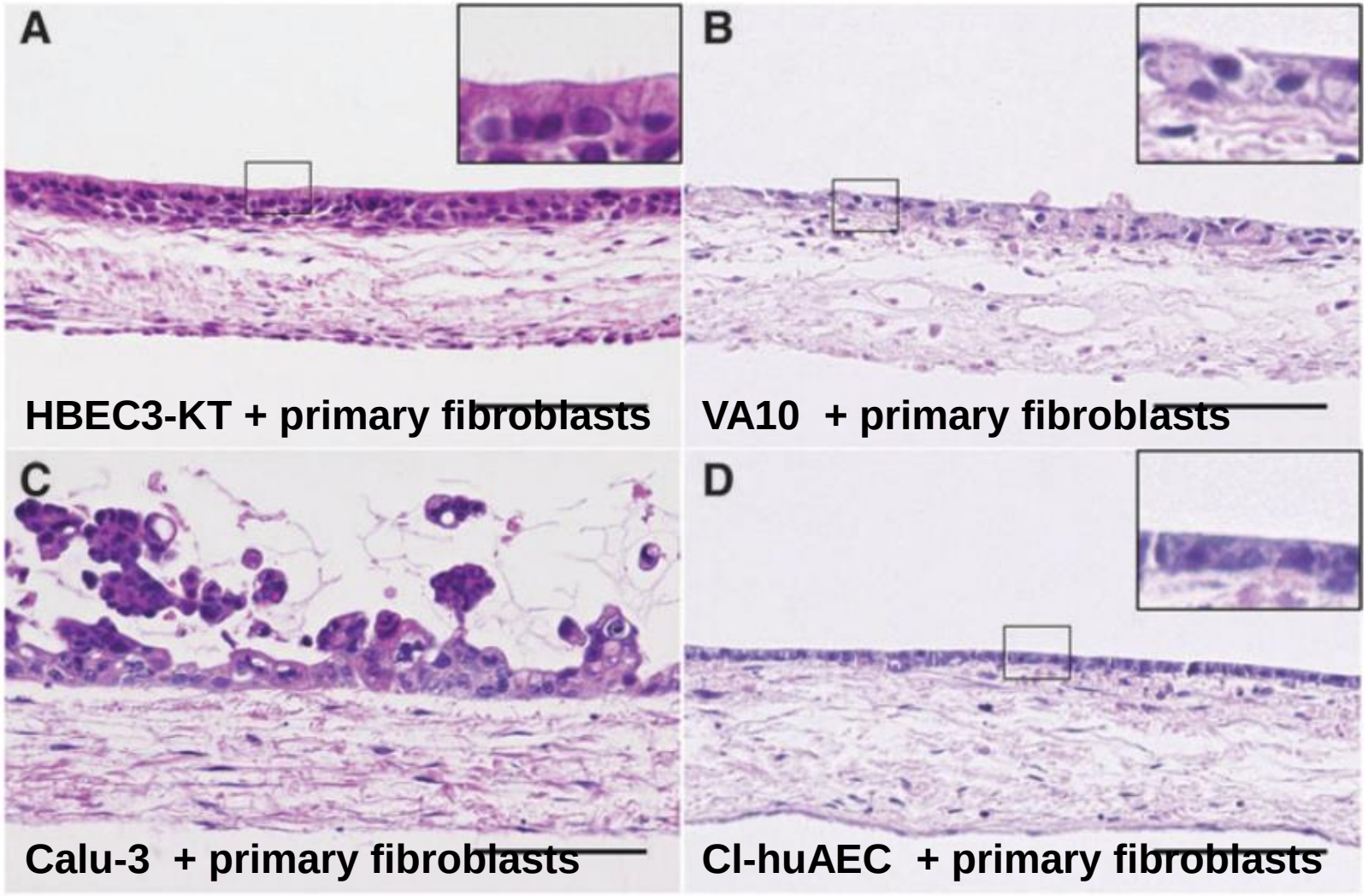
- Donor variance
- Donor bias (Disease as indication for surgery)
- Low passaging capability
- Access to primary tissue necessary

Advantages

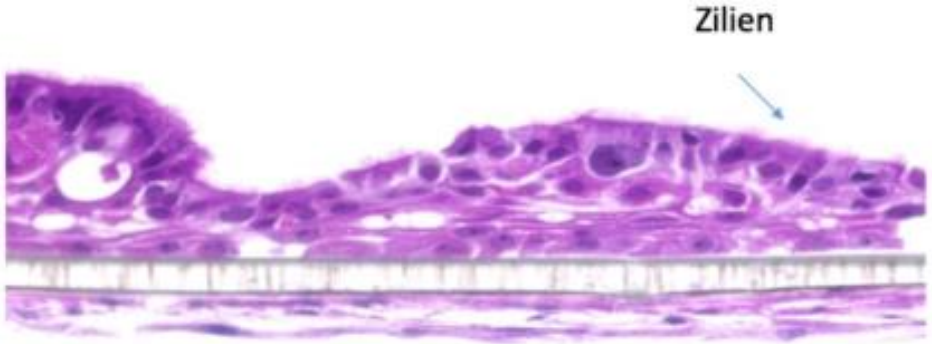
- Donor variance
- High *in vitro* – *in vivo* correlation
- Functionality
- Morphology
- Physiology (mucus)

- Possibility for disease specific models
- Personalized medicine

Use of primary cells – an advantage?



Lodes, et. al.; 2020



Meyer, et. al.; 2023

Primary human airway epithelial cells + autologous fibroblasts

Use of primary cells – an advantage?

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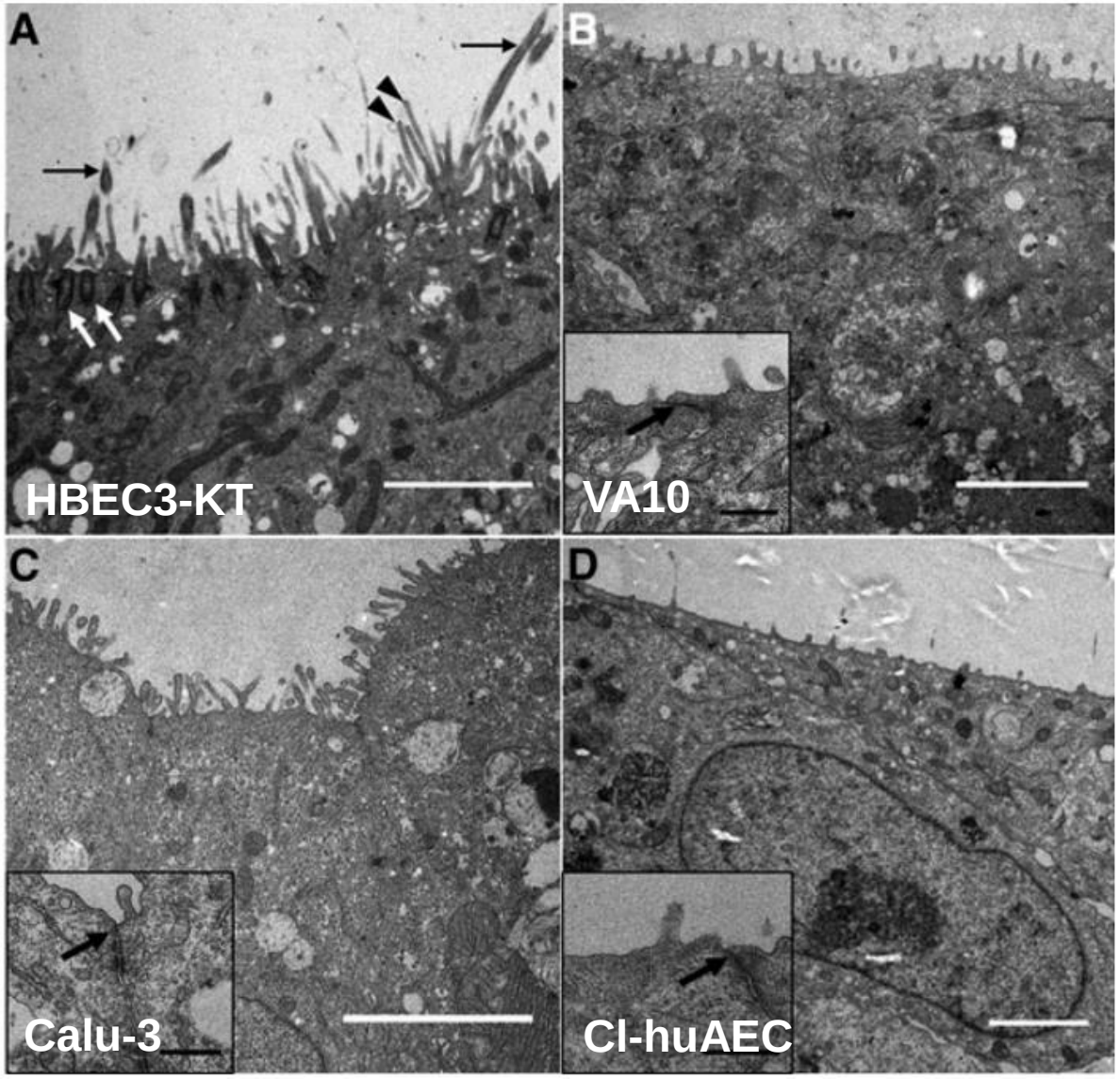
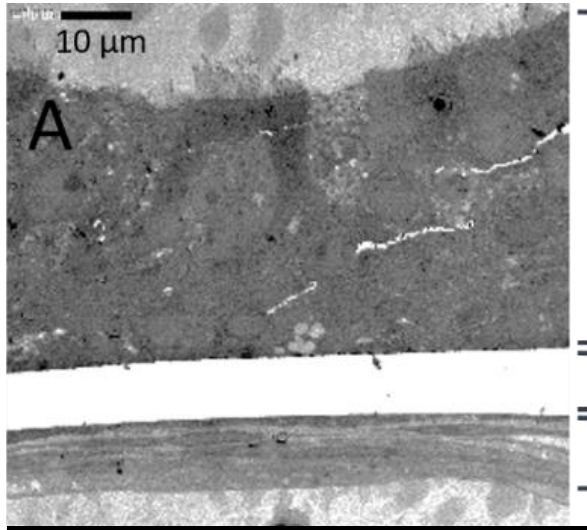


Lodes, et. al.; 2020



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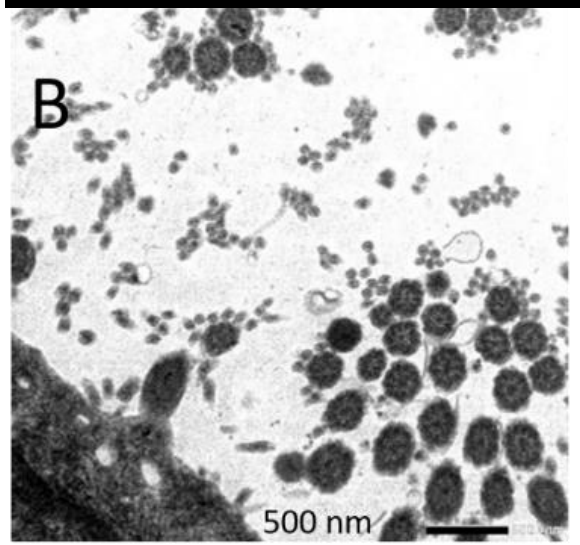

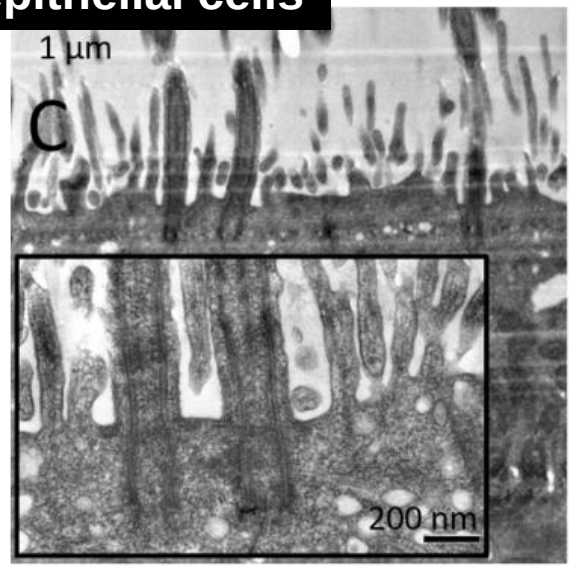

Use of primary cells – an advantage?

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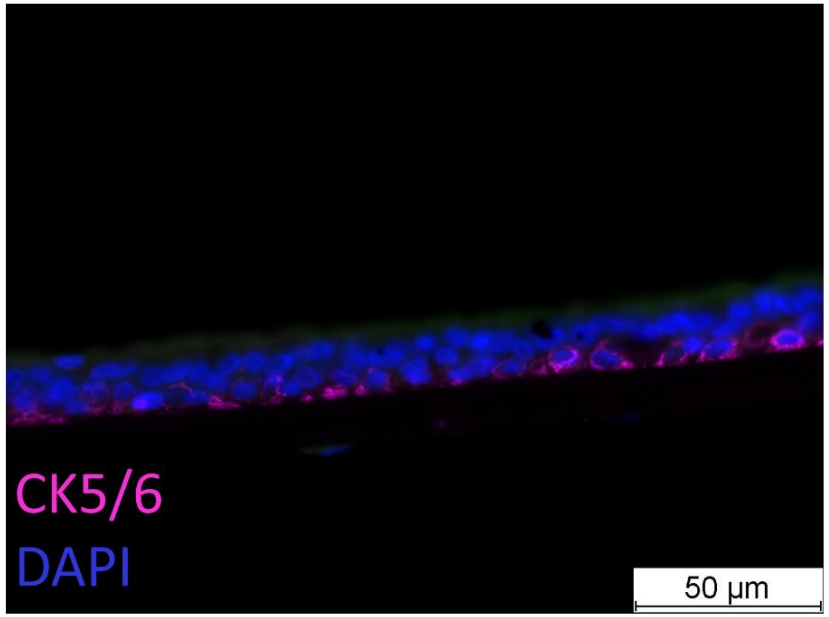

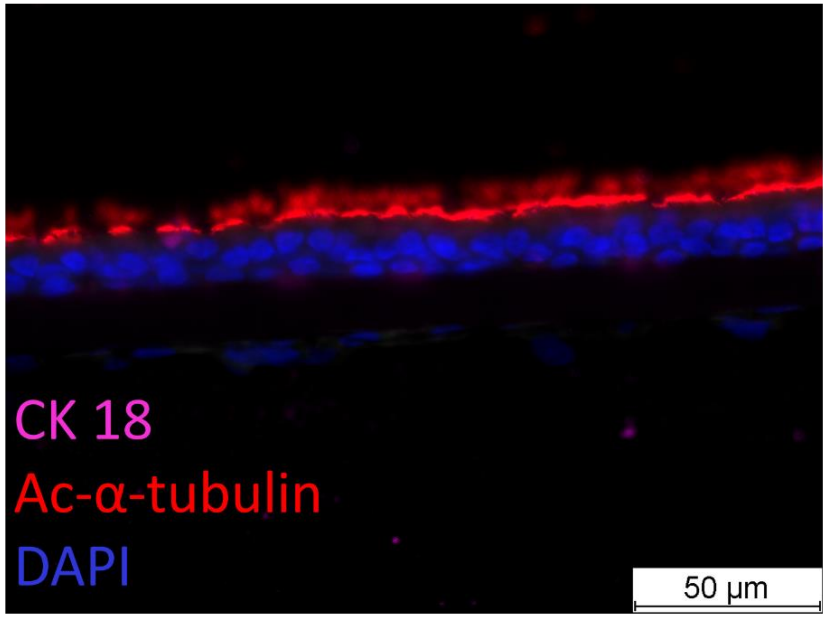

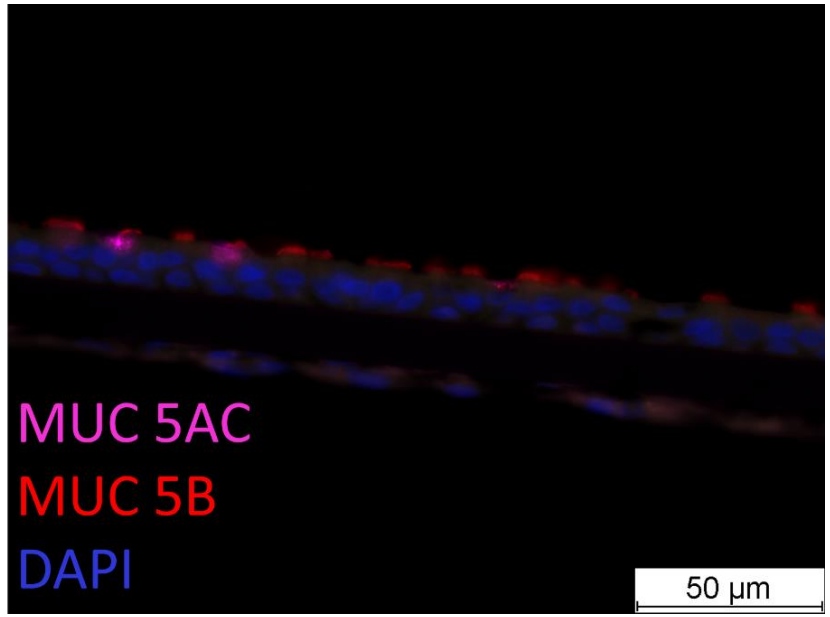

RNAsec of the respiratory mucosa models

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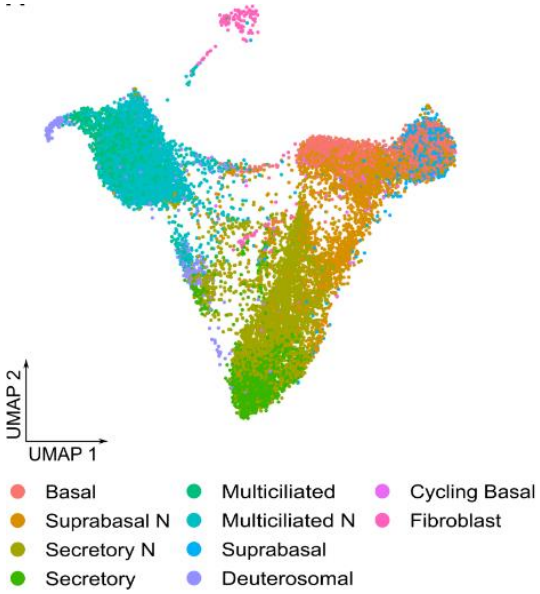

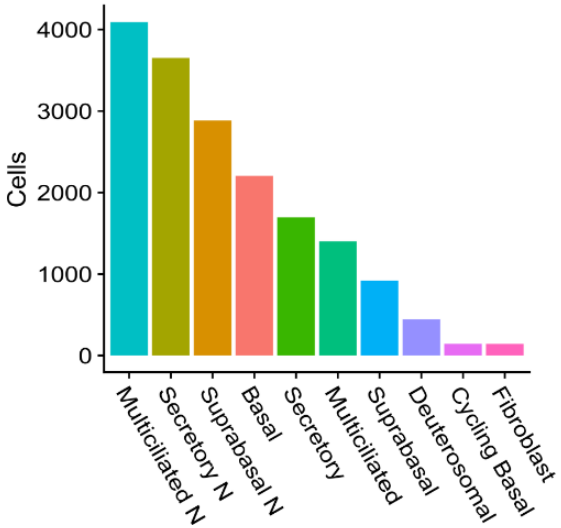

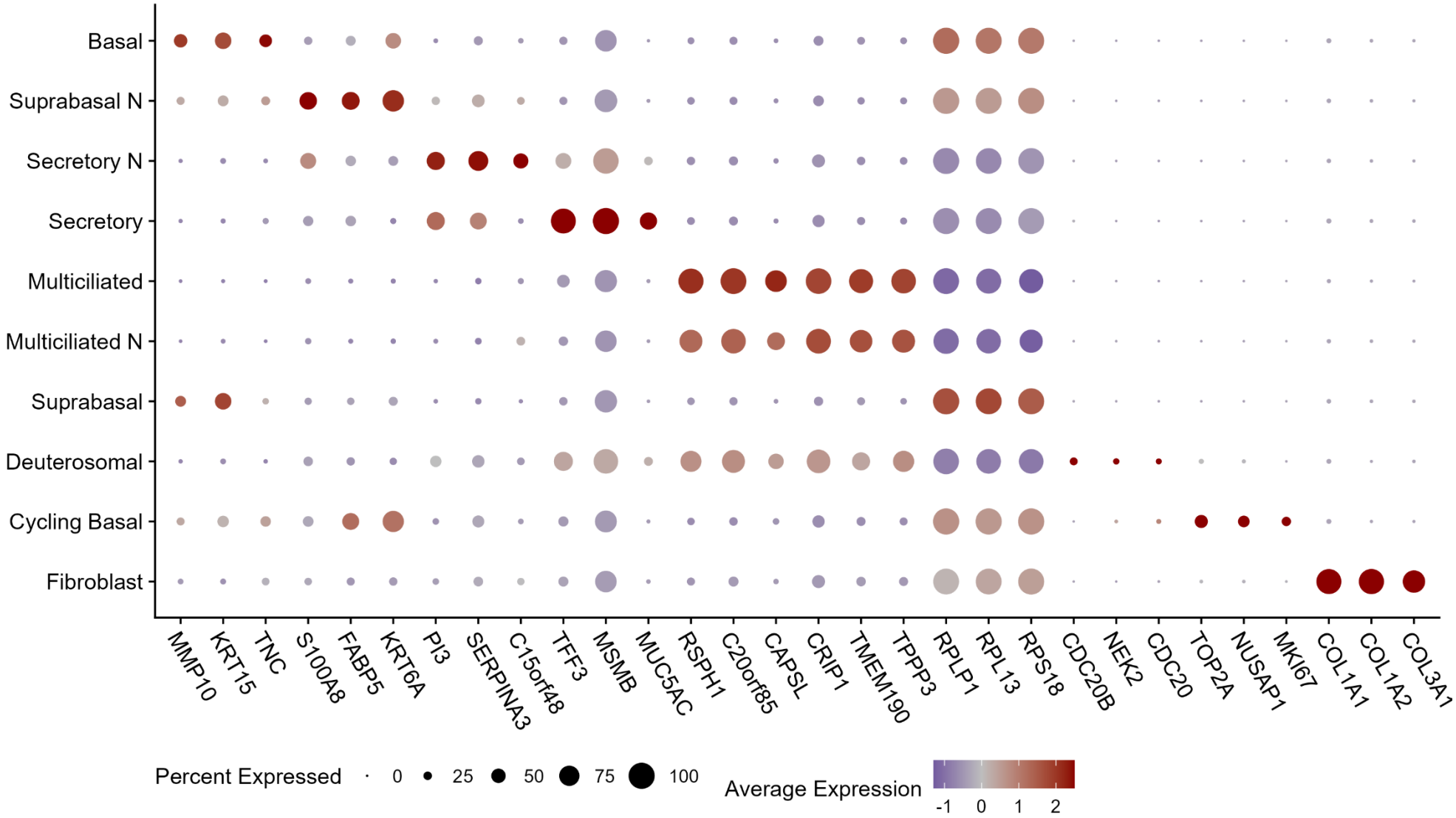

Merignac-Lacombe, et al.; 2024

Considerations in model establishment
Patient material – how heterogenous is it?

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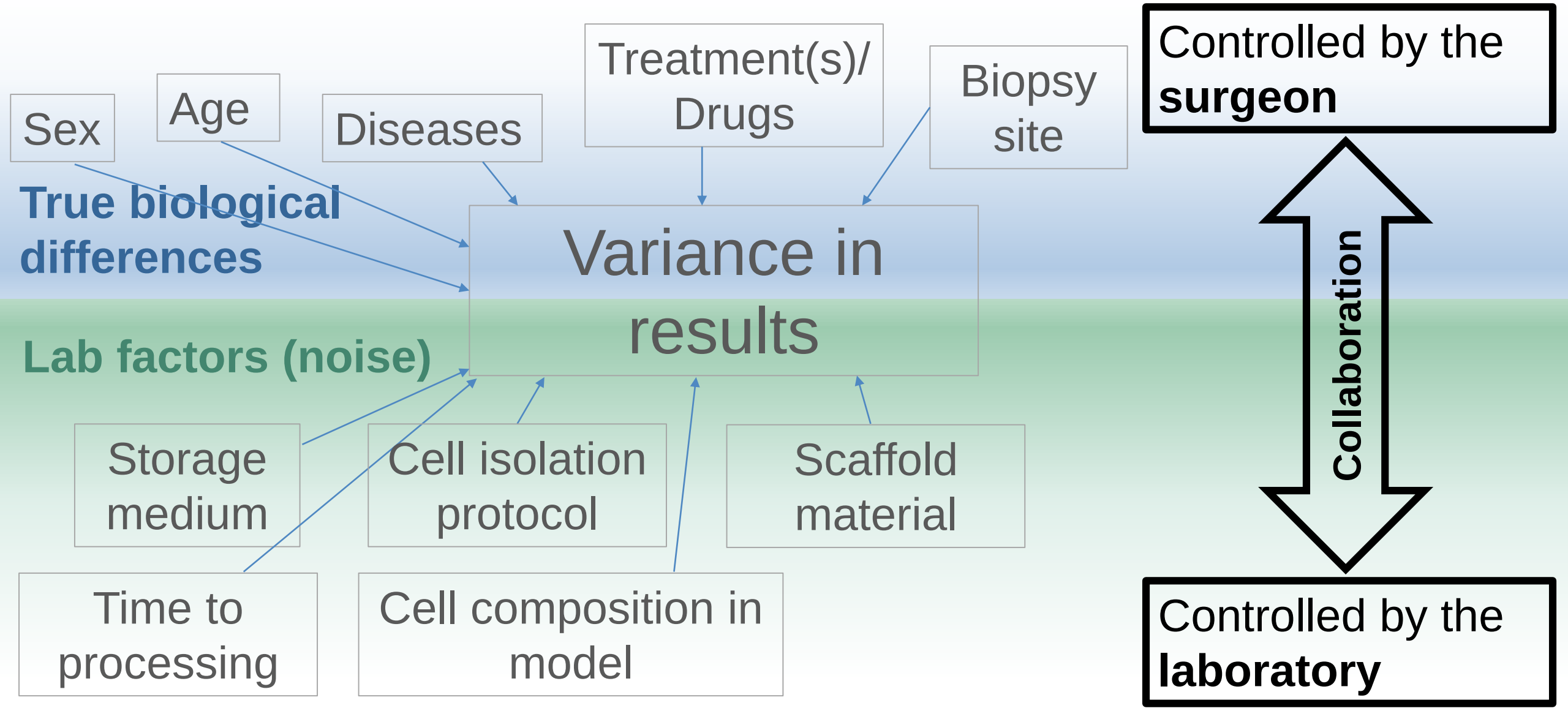

Impact of UFP on respiratory mucosa models

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Characterization of the interaction between UFP and cells of the respiratory tract in an **offline** experiment

Characterization of the interaction between UFP and cells of the respiratory tract in an **online** experiment

Using **standardized** ultrafine particles
Carbon Black - Printex 90

Use **live generated** particles by partners with clear characterization
Propane combustion

BAY UFP
BAYERISCHER PROJEKTVERBUND ULTRAFEINE PARTIKEL

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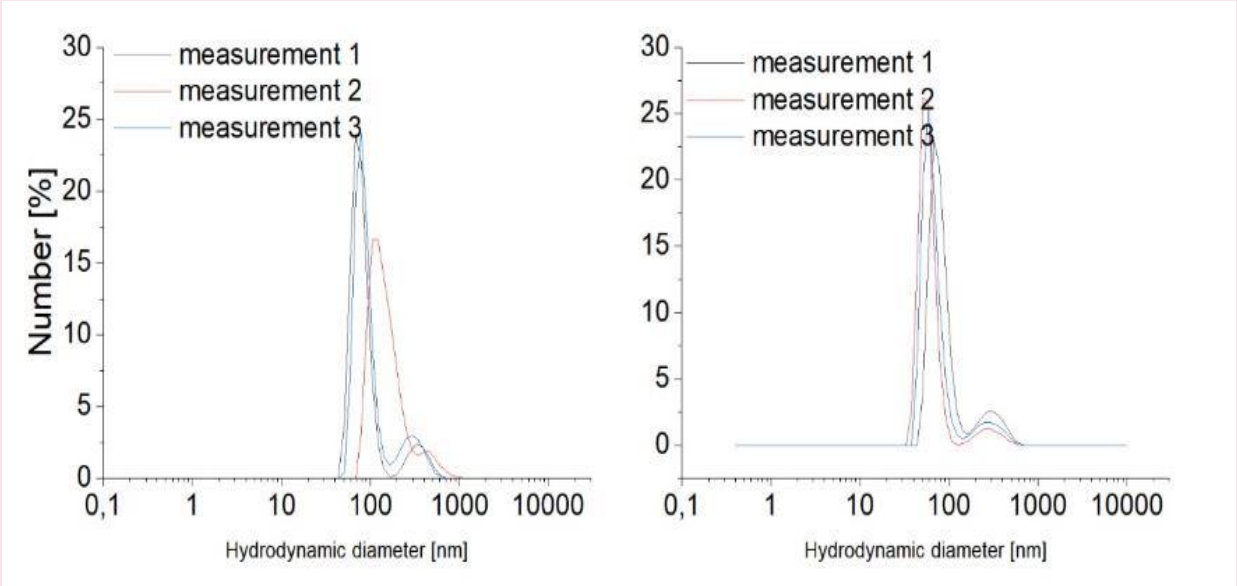
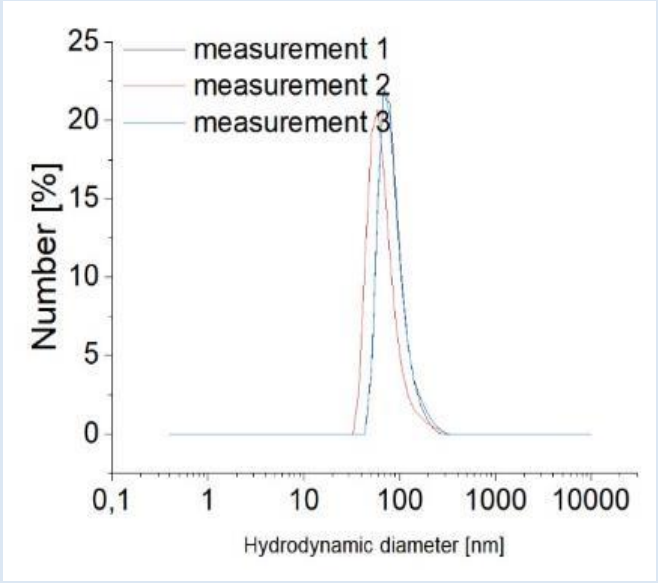
Bayerisches Staatsministerium für Umwelt und Verbraucherschutz

UFP characterization, Carbon Black – Printex 90



Experimental contisions plus 1 hour

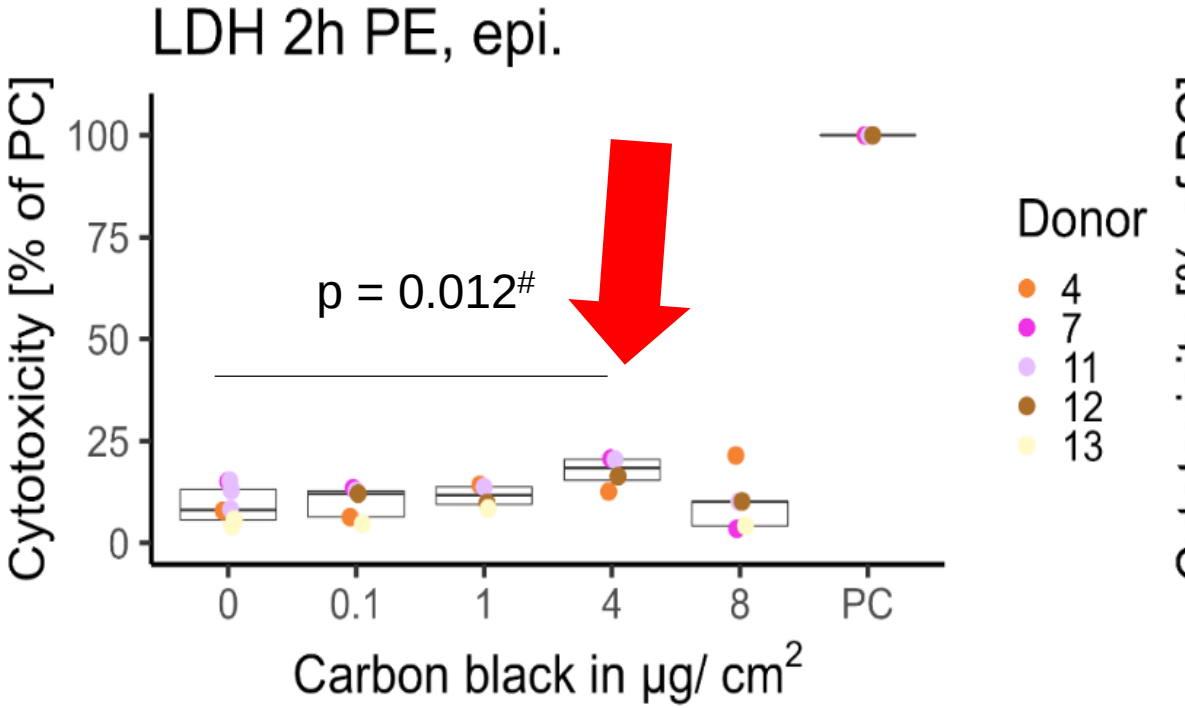
Additional sonification 1 minute before measurement

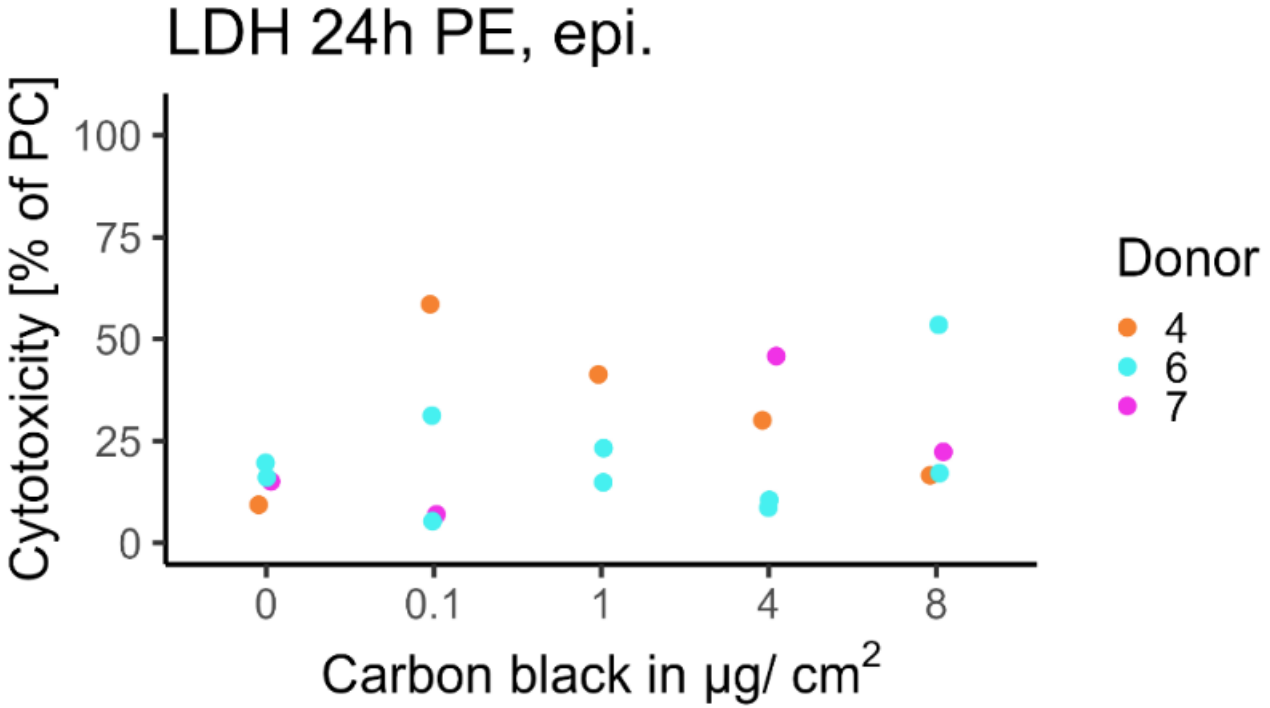


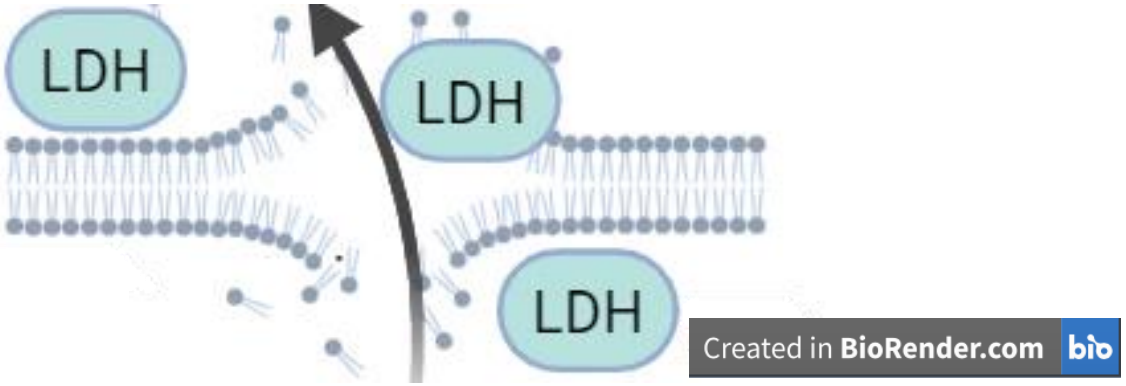
In water, sonification 1 minute before measurement

- Particle size like expected
- Particle agglomerates in dispersion, more agglomerates in cell culture medium

Cytotoxicity after Carbon Black exposition

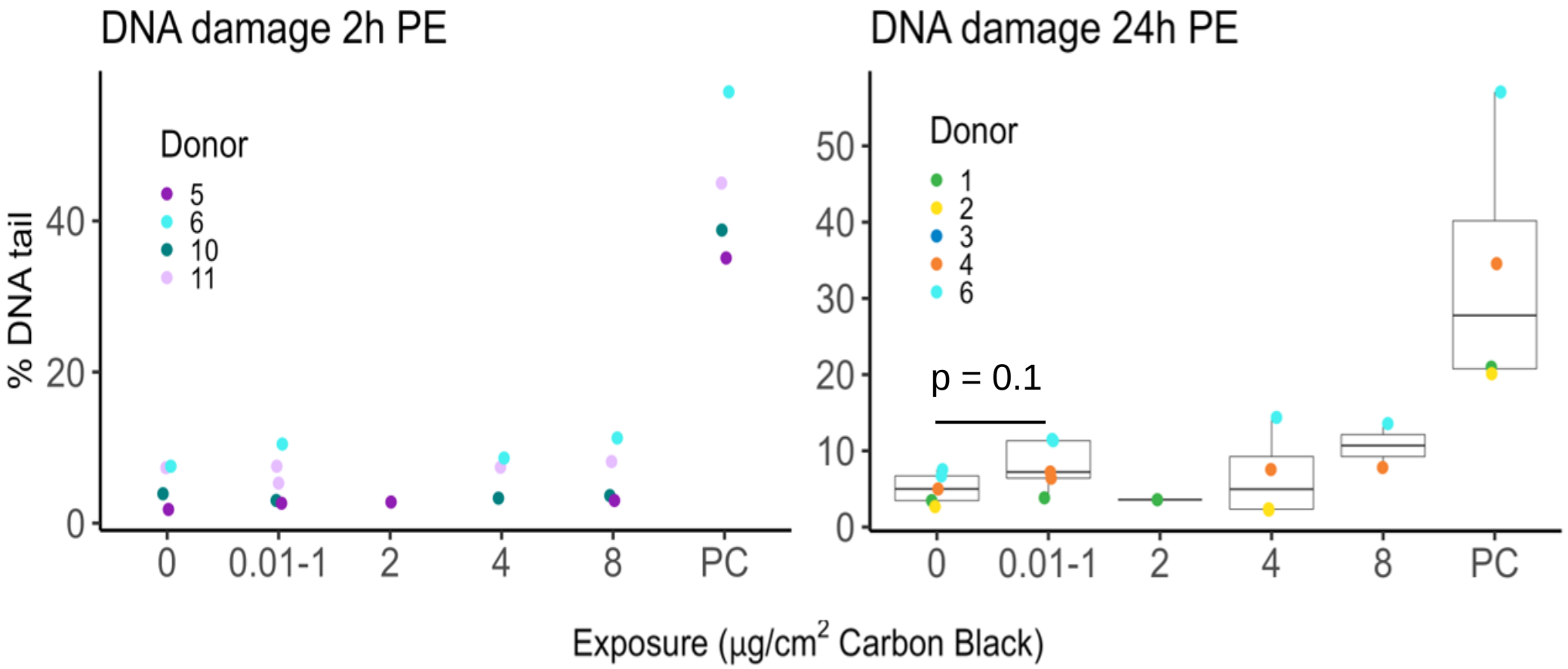


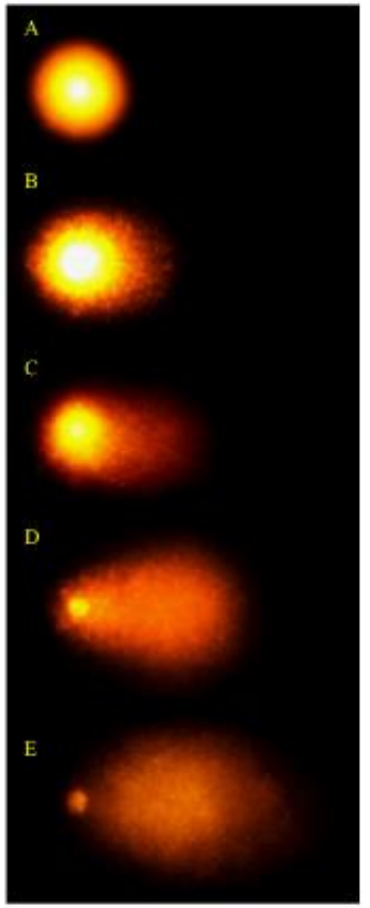




- Significant difference
- No cytotoxicity

DNA-damage after Carbon Black exposition





- No genotoxic response at investigated timepoints
- Donor tendencies (repair?)

Cytokine release after Carbon black exposition

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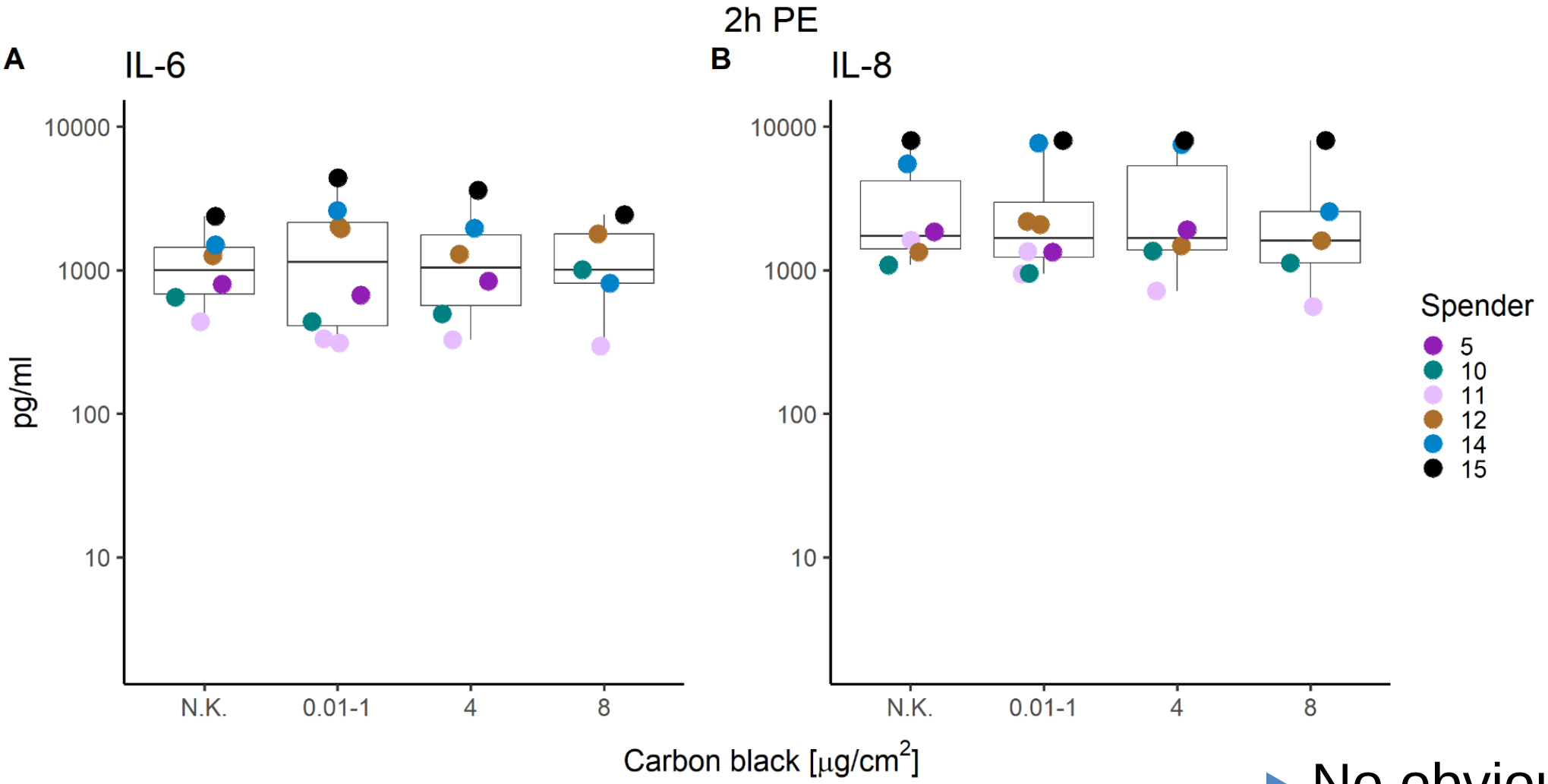

- No obvious response
- Donor tendeinces

Barrier integrity after Carbon Black exposition

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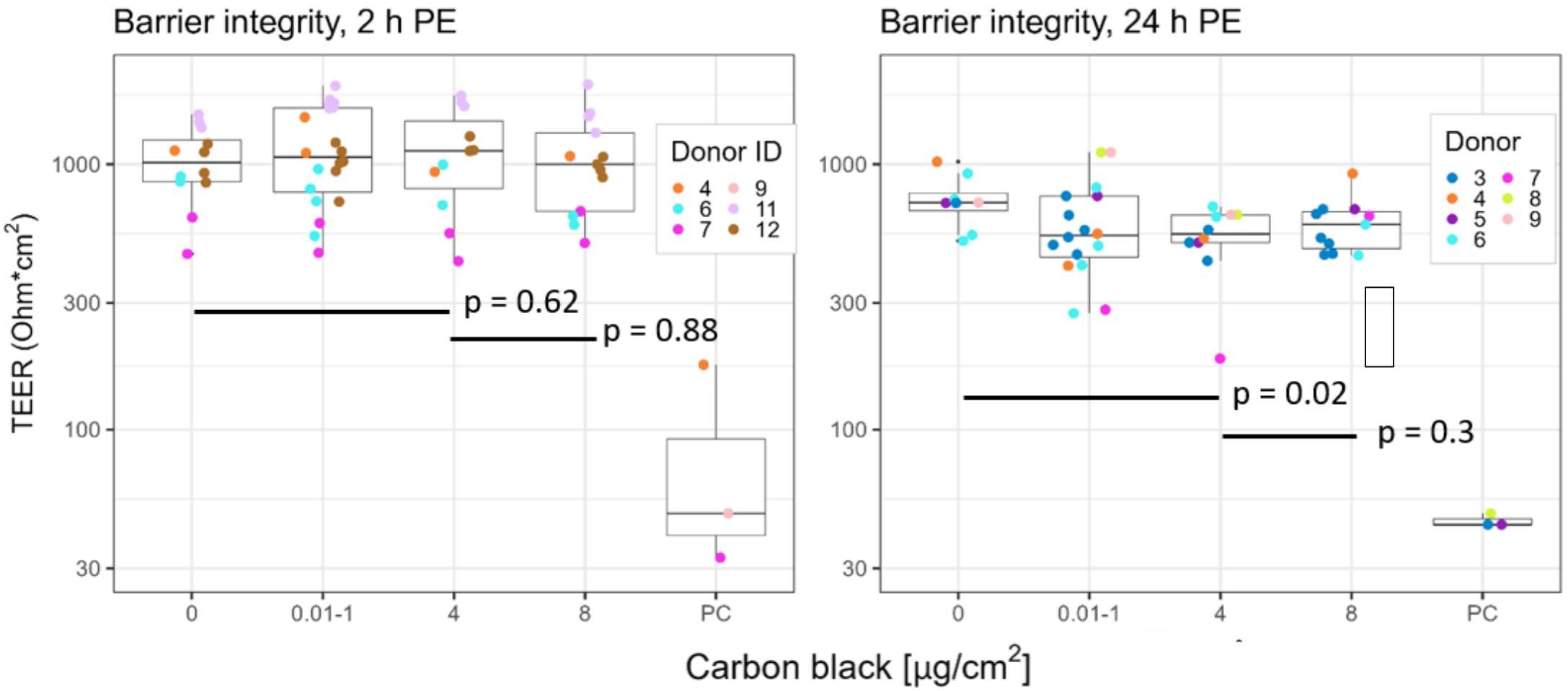

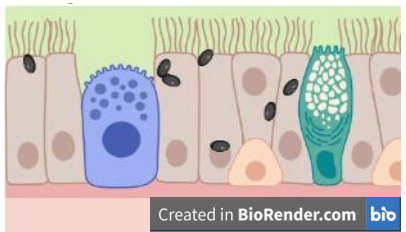

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Intracellular without specific localization

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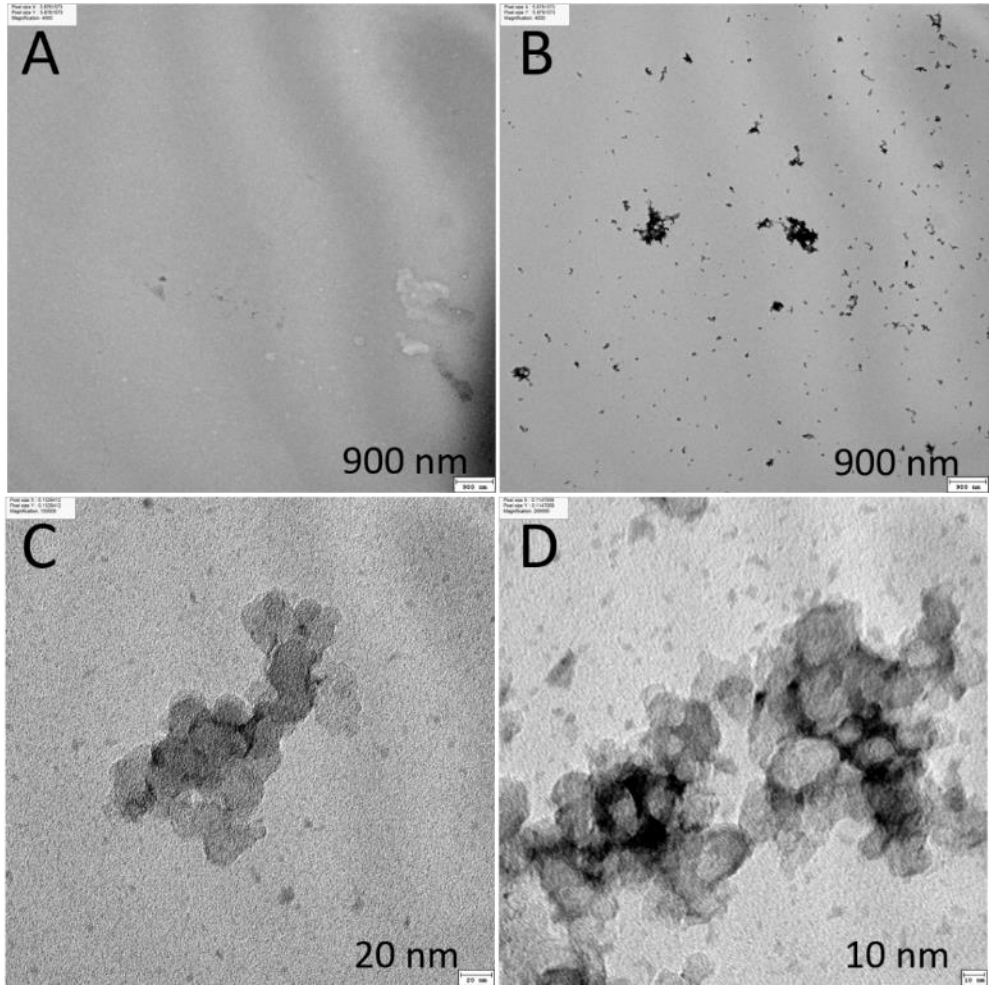
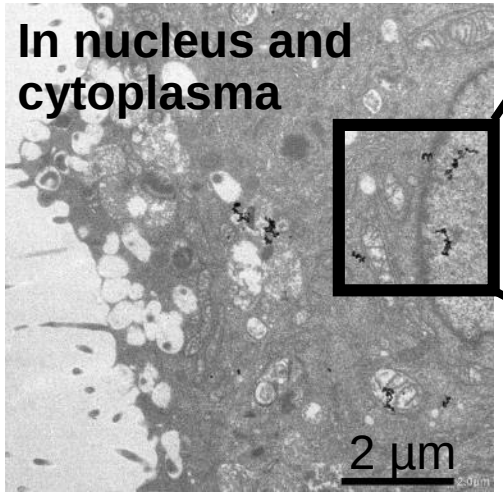
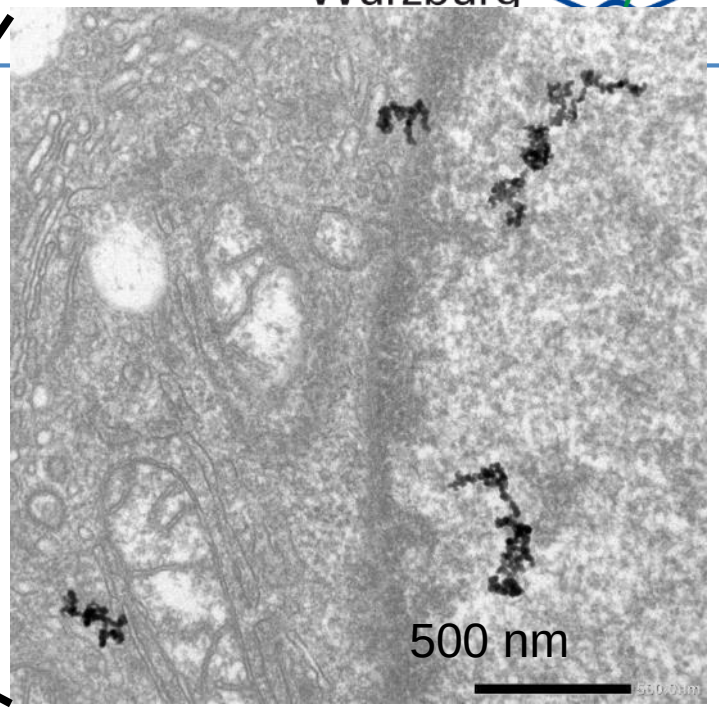
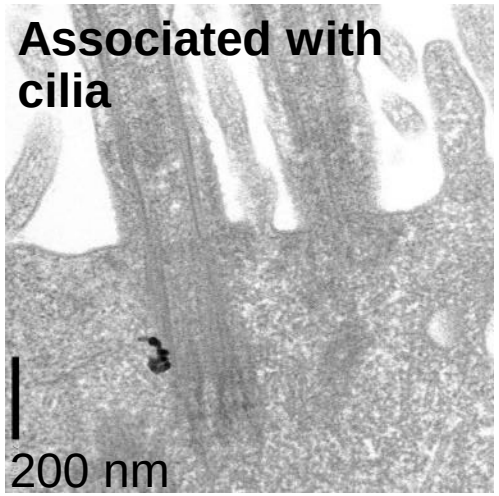
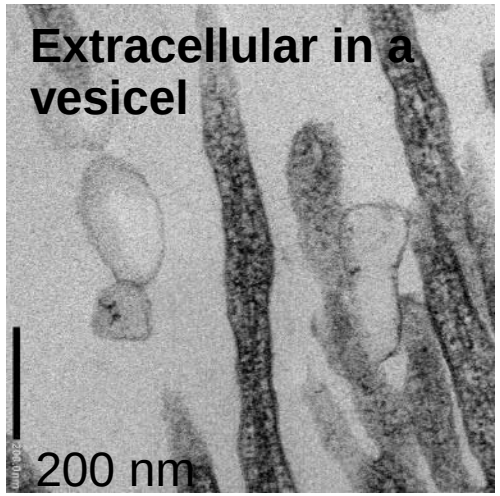
Carbon black in water











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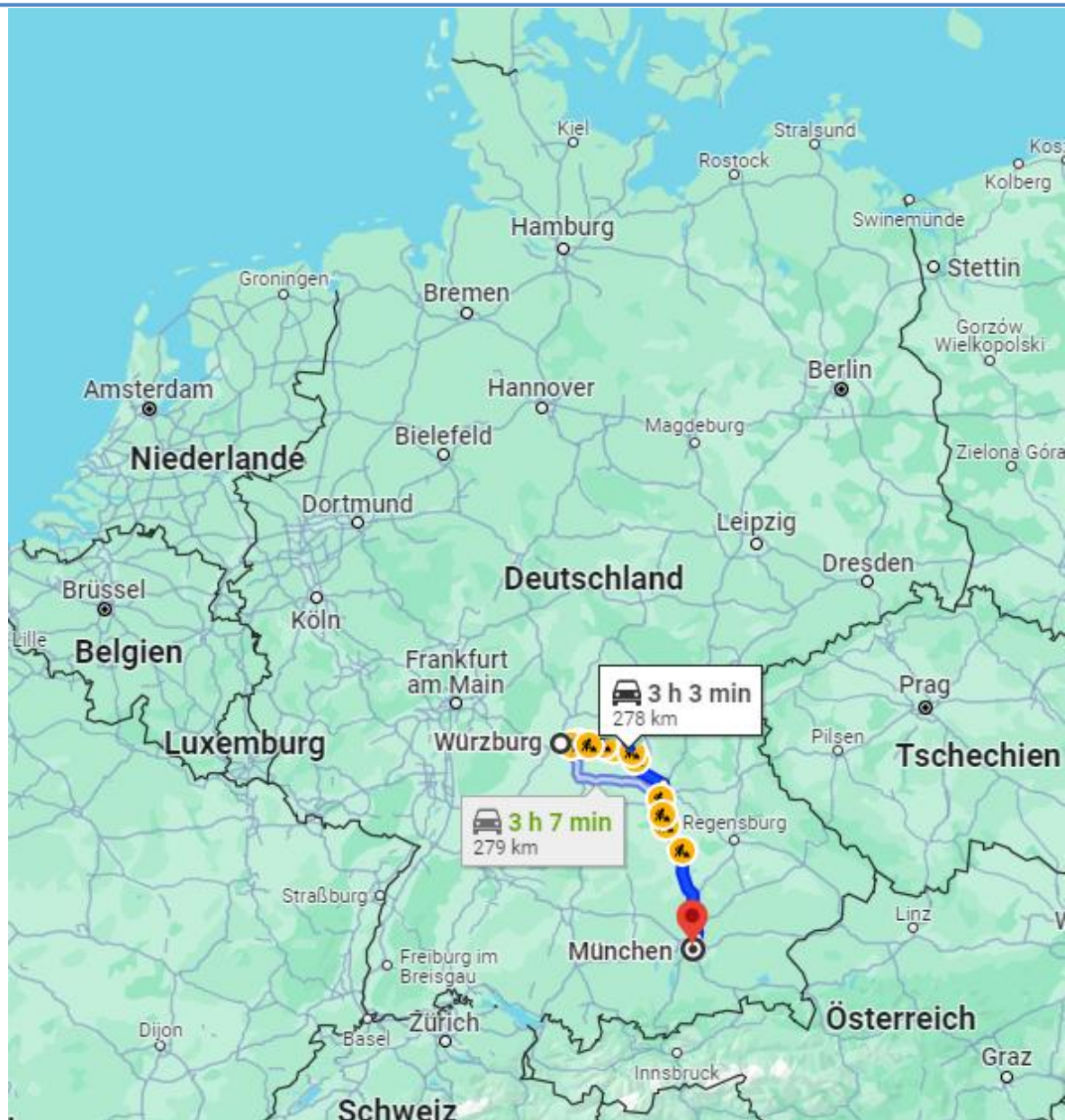
finanziert durch
Bayerisches Staatsministerium für Umwelt und Verbraucherschutz

UFP online exposition in Munich

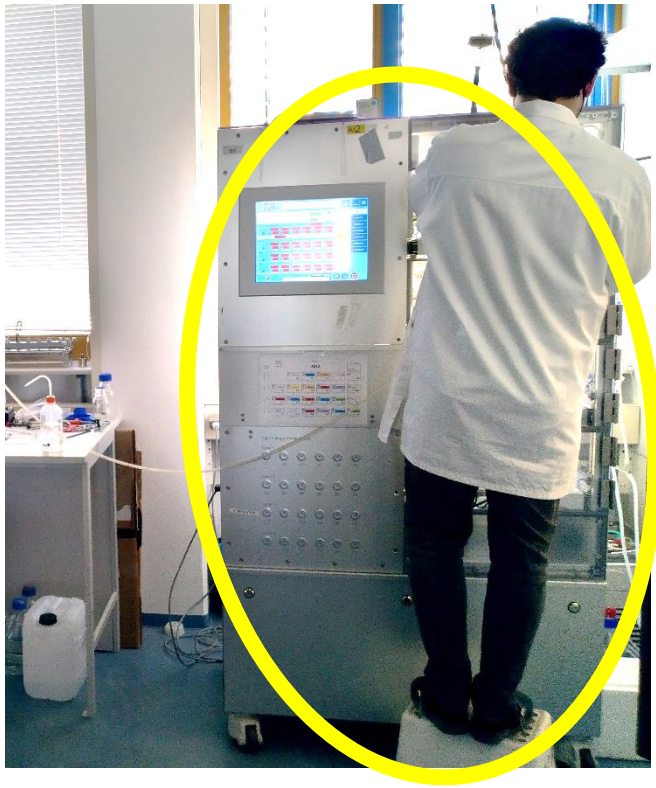
Uniklinikum Würzburg

Involved partners in Munich

- Anja Huber
- Anusmita Das
- Elias Zimmermann
- Evelyn Kuhn
- Jana Pantzke
- Johannes Becker
- Martin Sklorz
- Mathilde Delaval
- Mohammad Almasaleekh
- Ralf Zimmermann
- Sebastiano Di Bucchianico
- Seongh Jeong
- Svenja Offer



Online exposures – compatible with commercial inserts



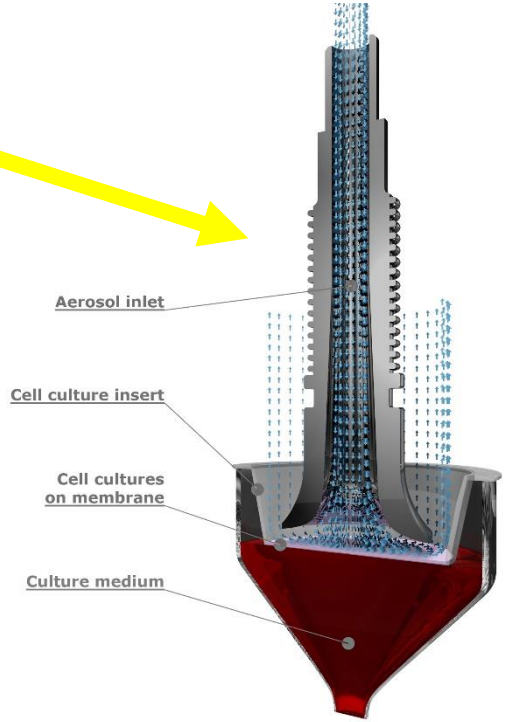
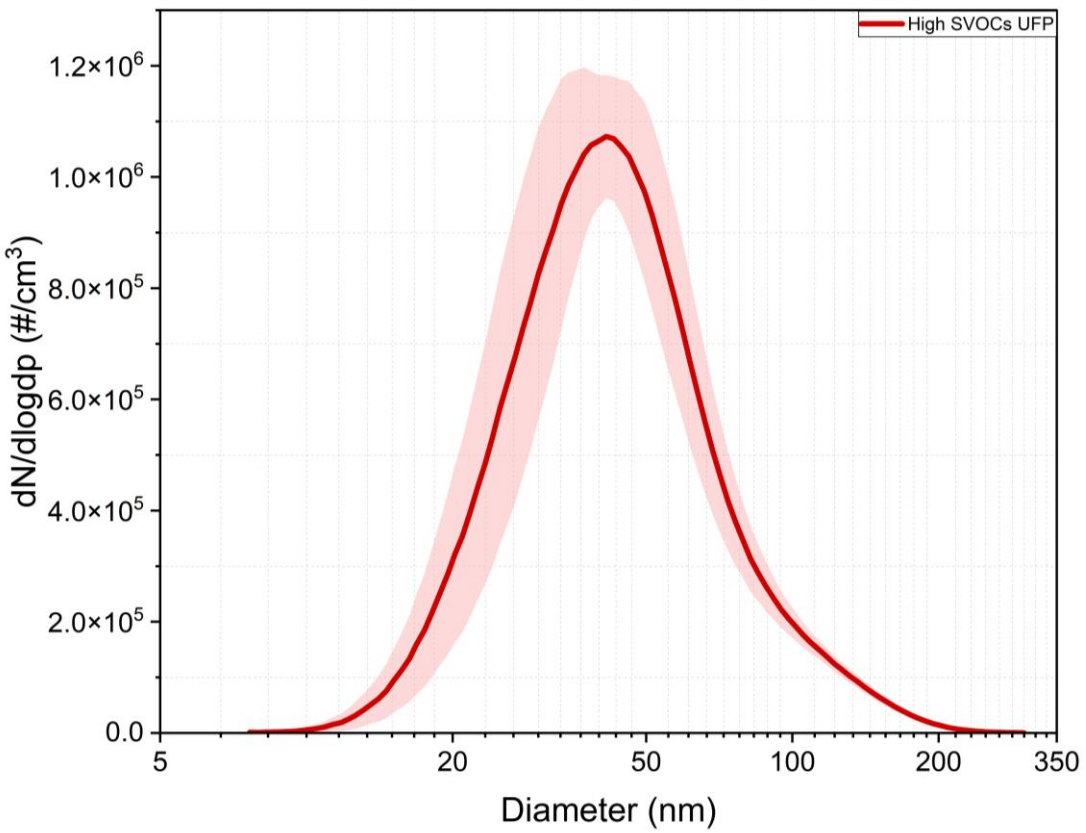
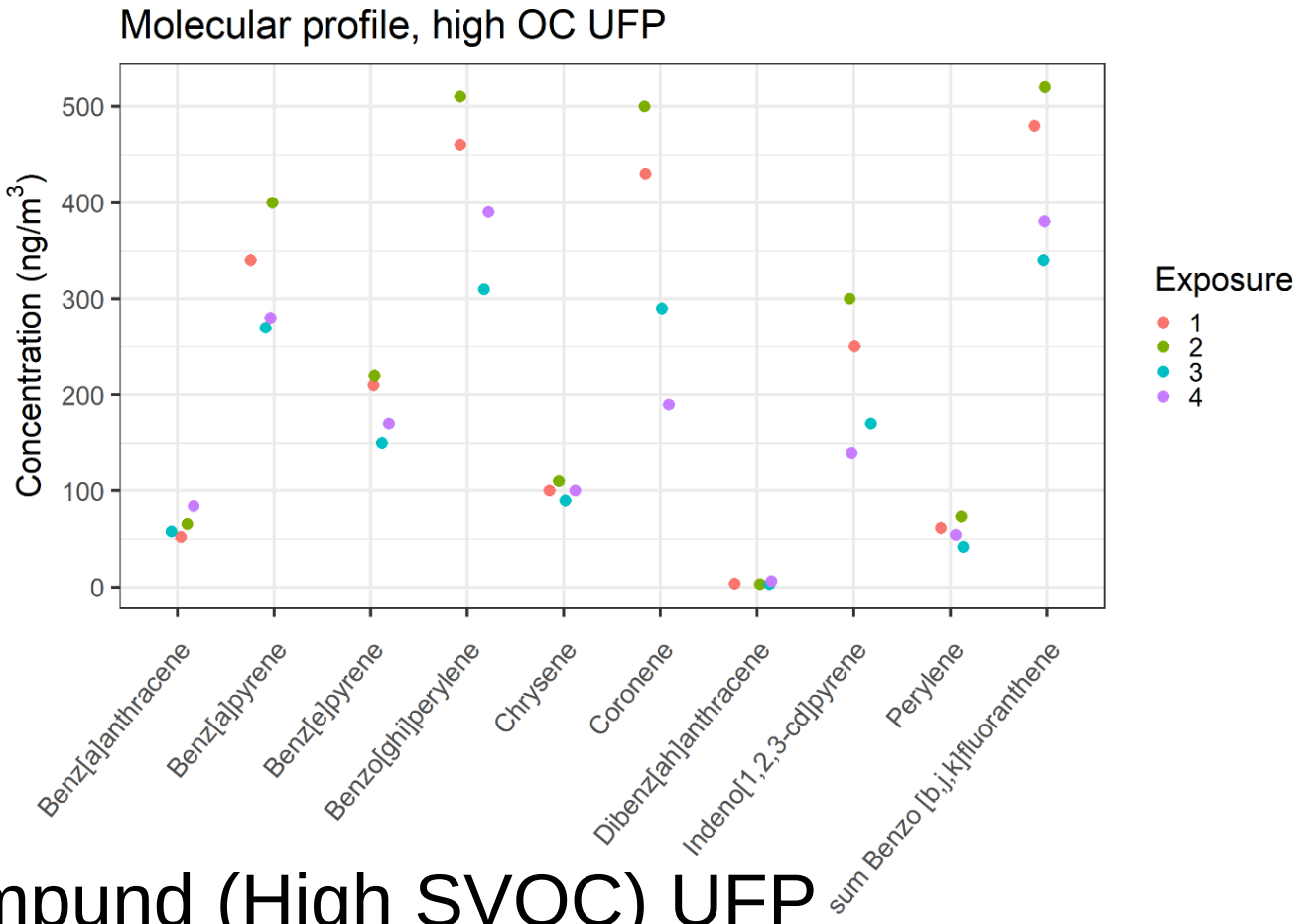


Image: Vitrocell (re-used with permission)

Online generated UFP-particles





- Semi-volatile high organic compund (High SVOC) UFP
- Combined data from 2022 and 2023
- Particle size peak ~45 nm

Morphology of the „low OC“-particles

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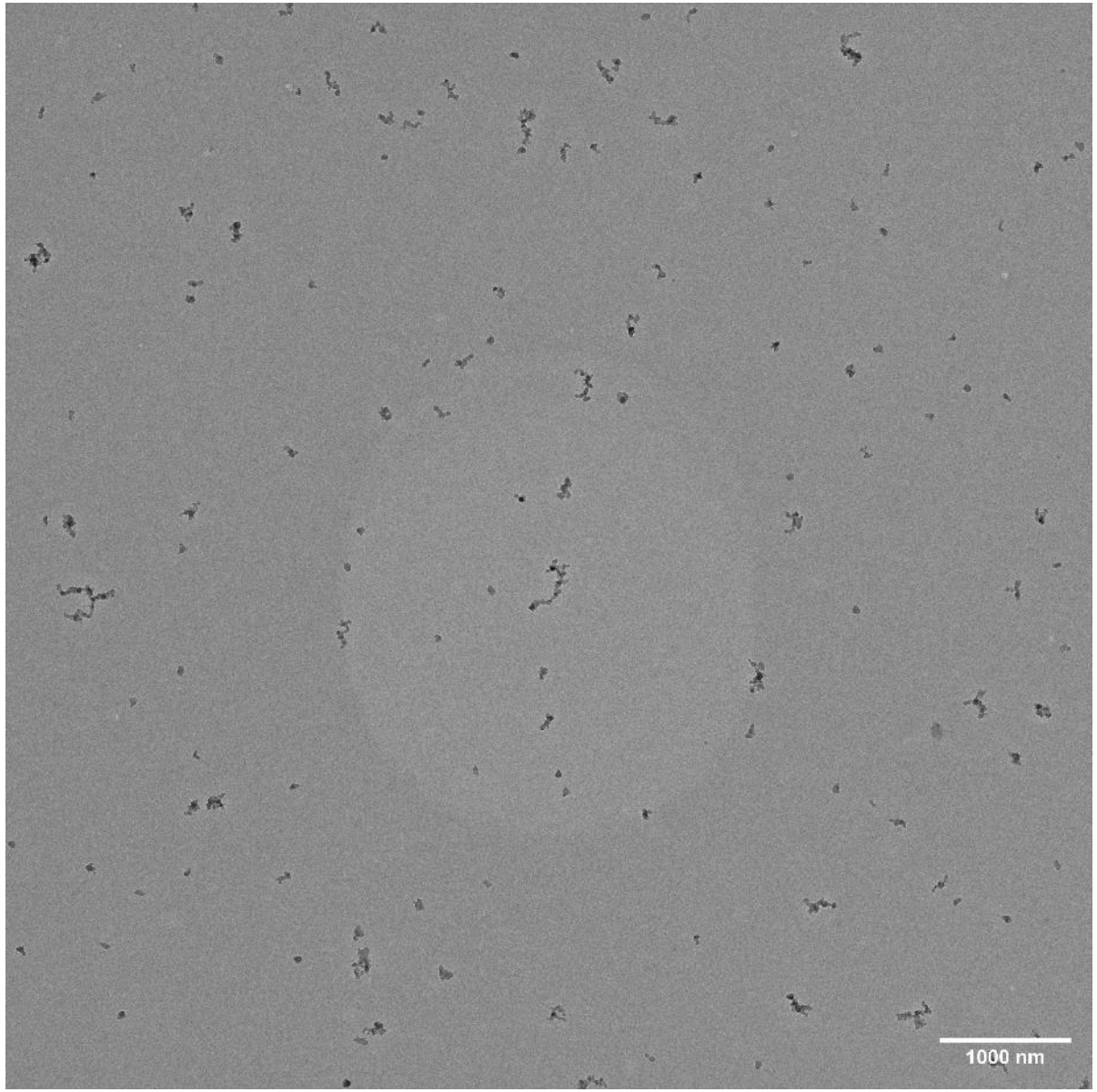

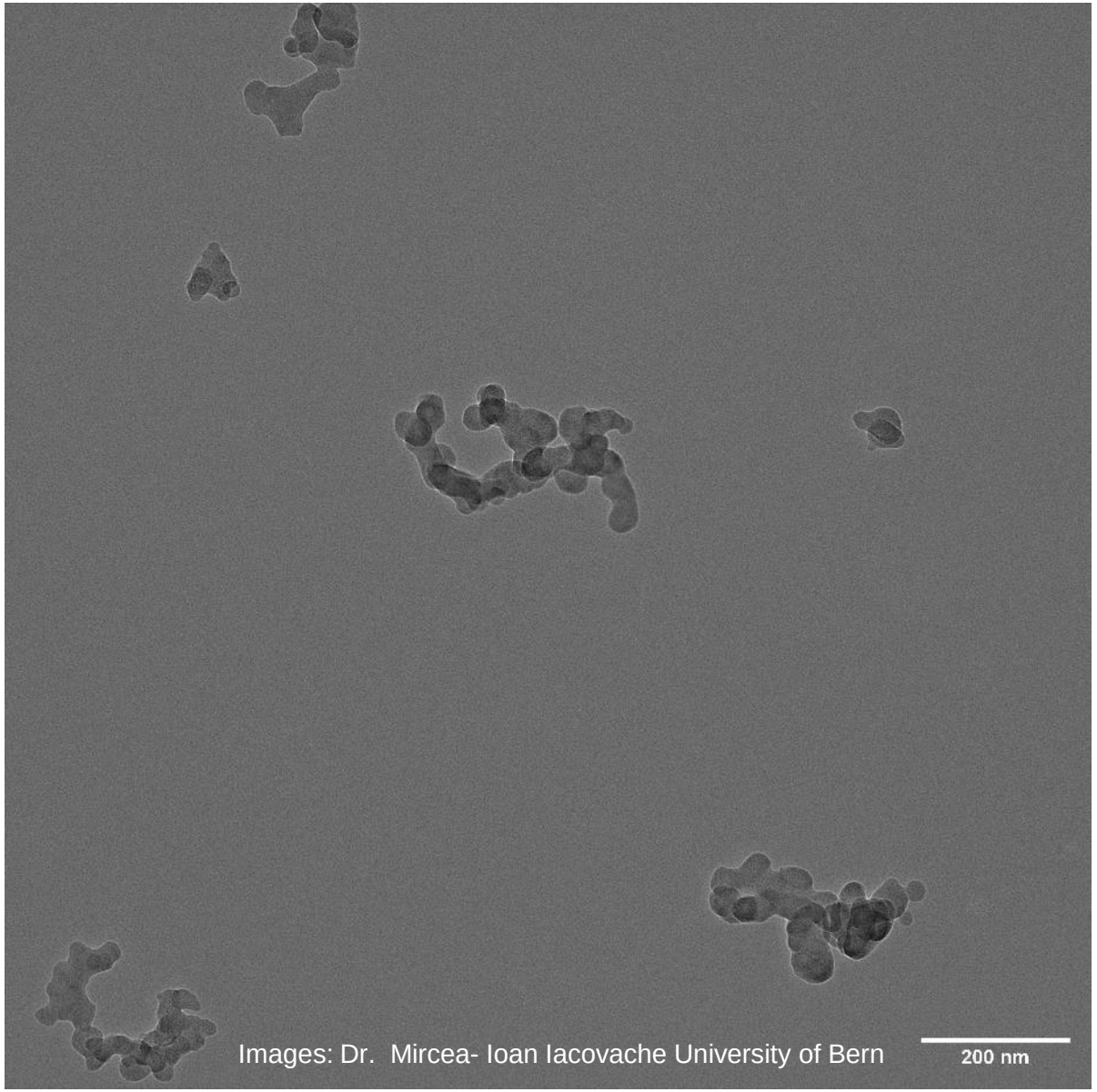

LDH release 2 hours after UFP online exposition

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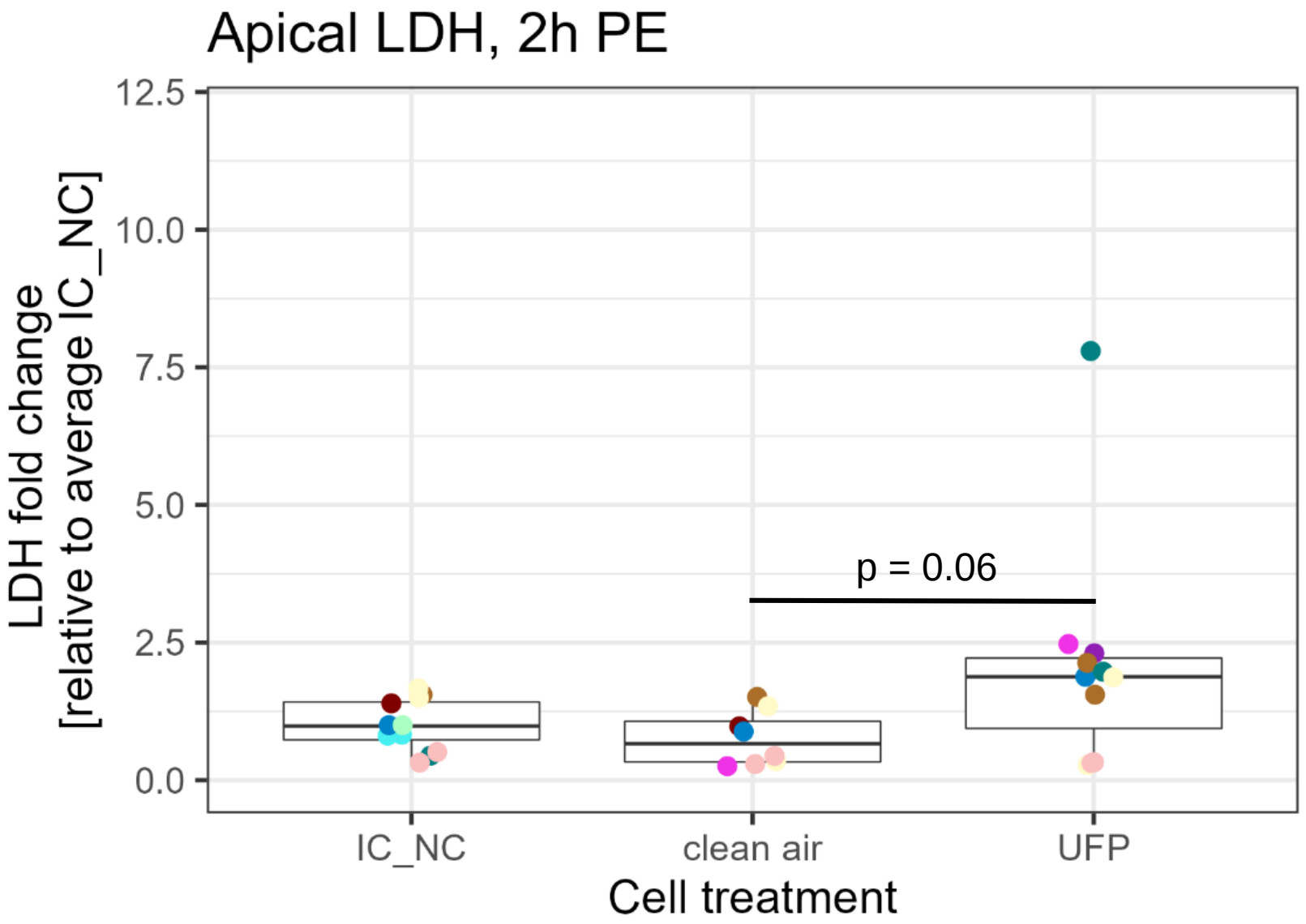
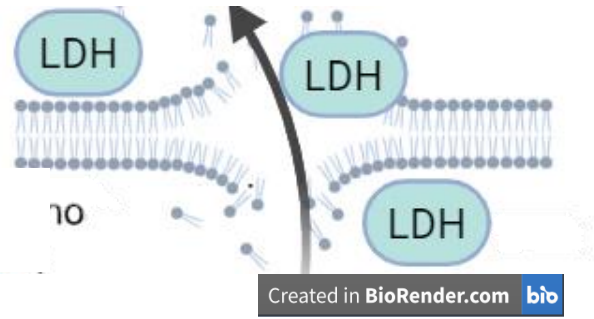
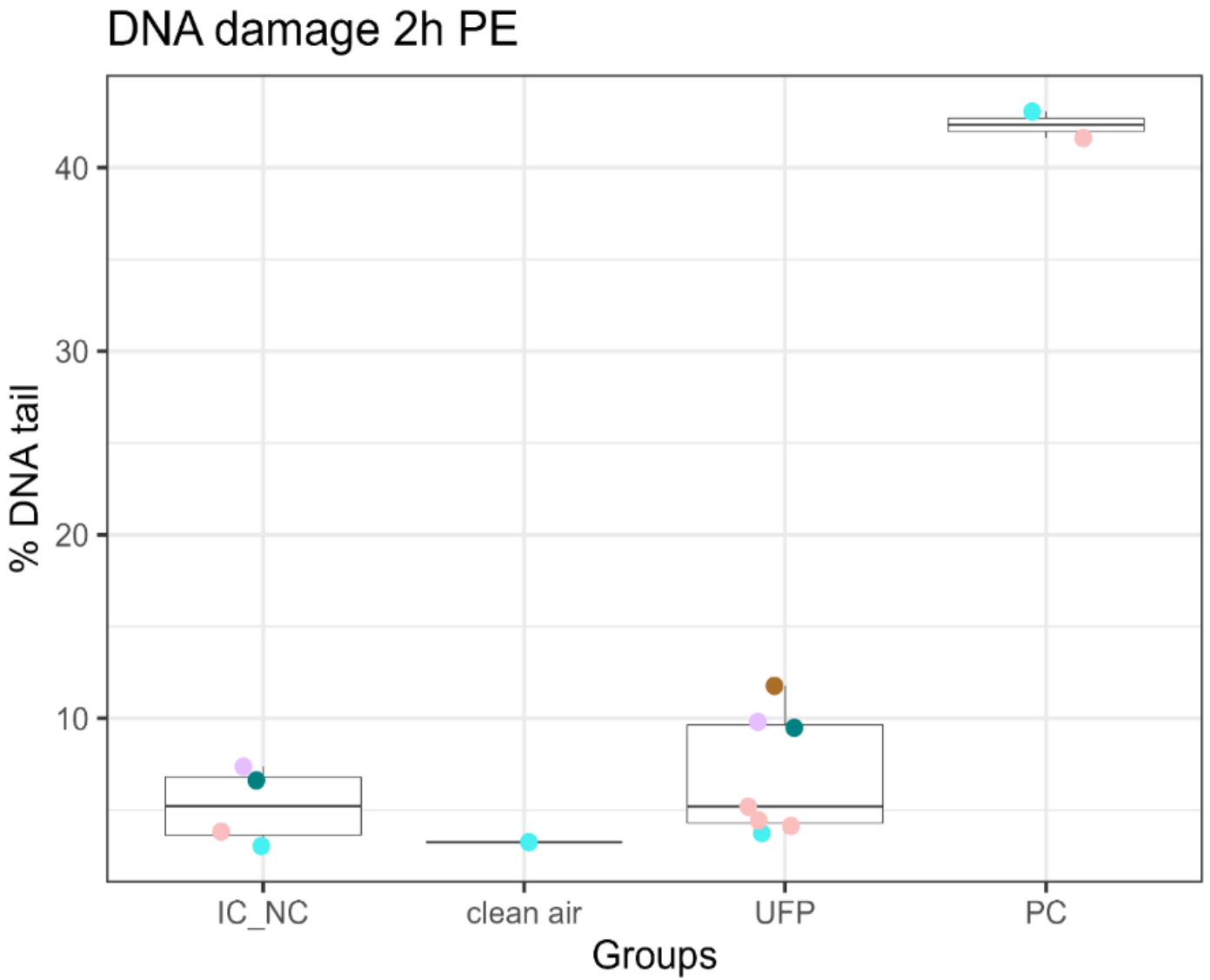
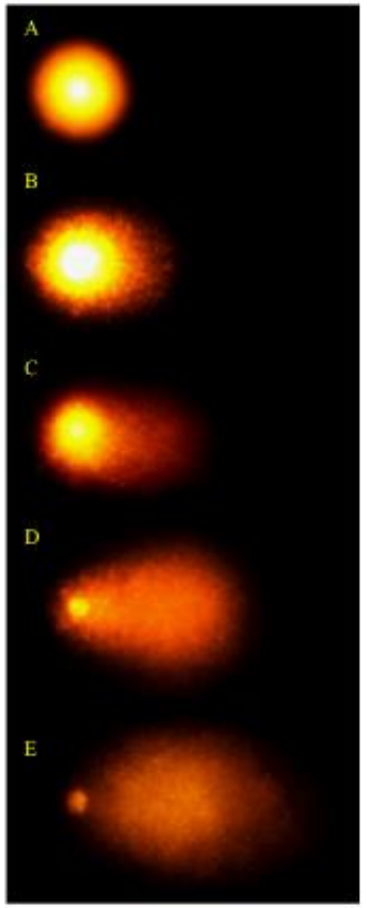




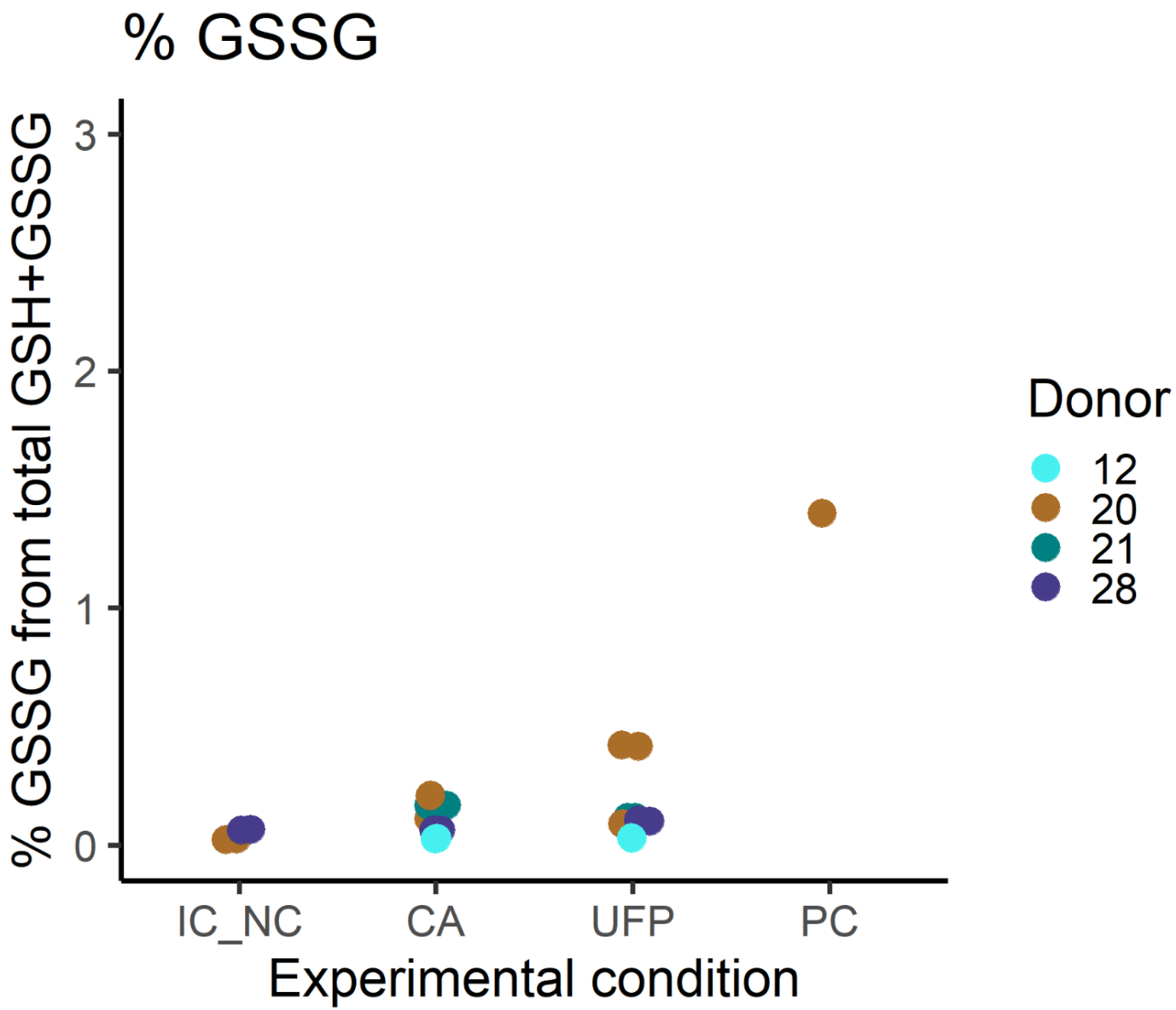
DNA-damage 2 h after UFP online exposition



Donor specific baseline?



Oxidative stress 2 h after UFP online exposition



Barrier integrity after UFP online exposition

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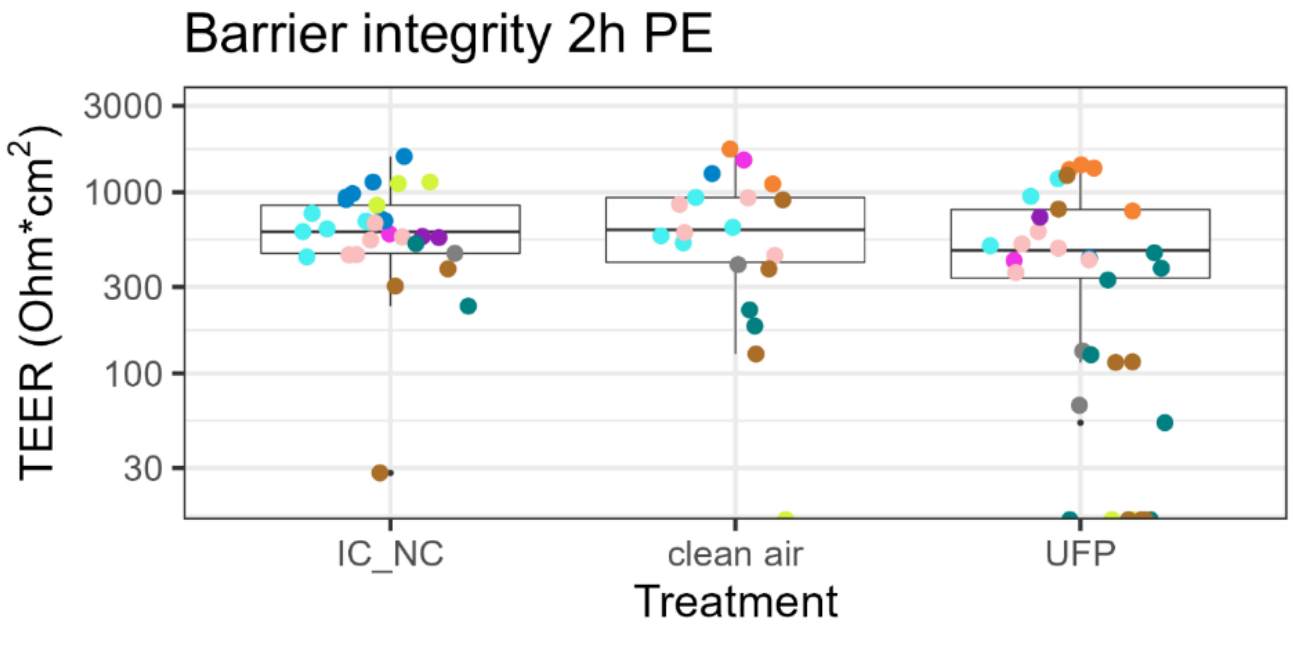

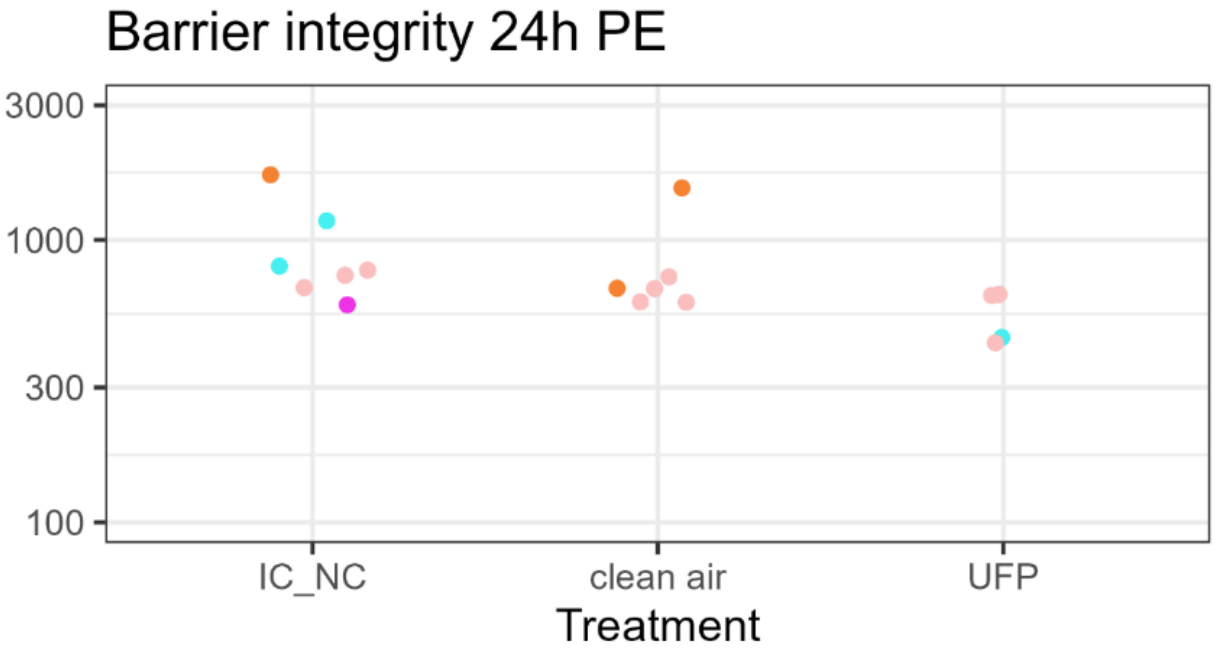

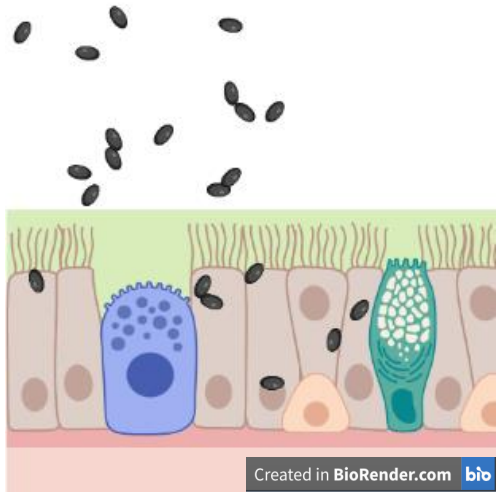

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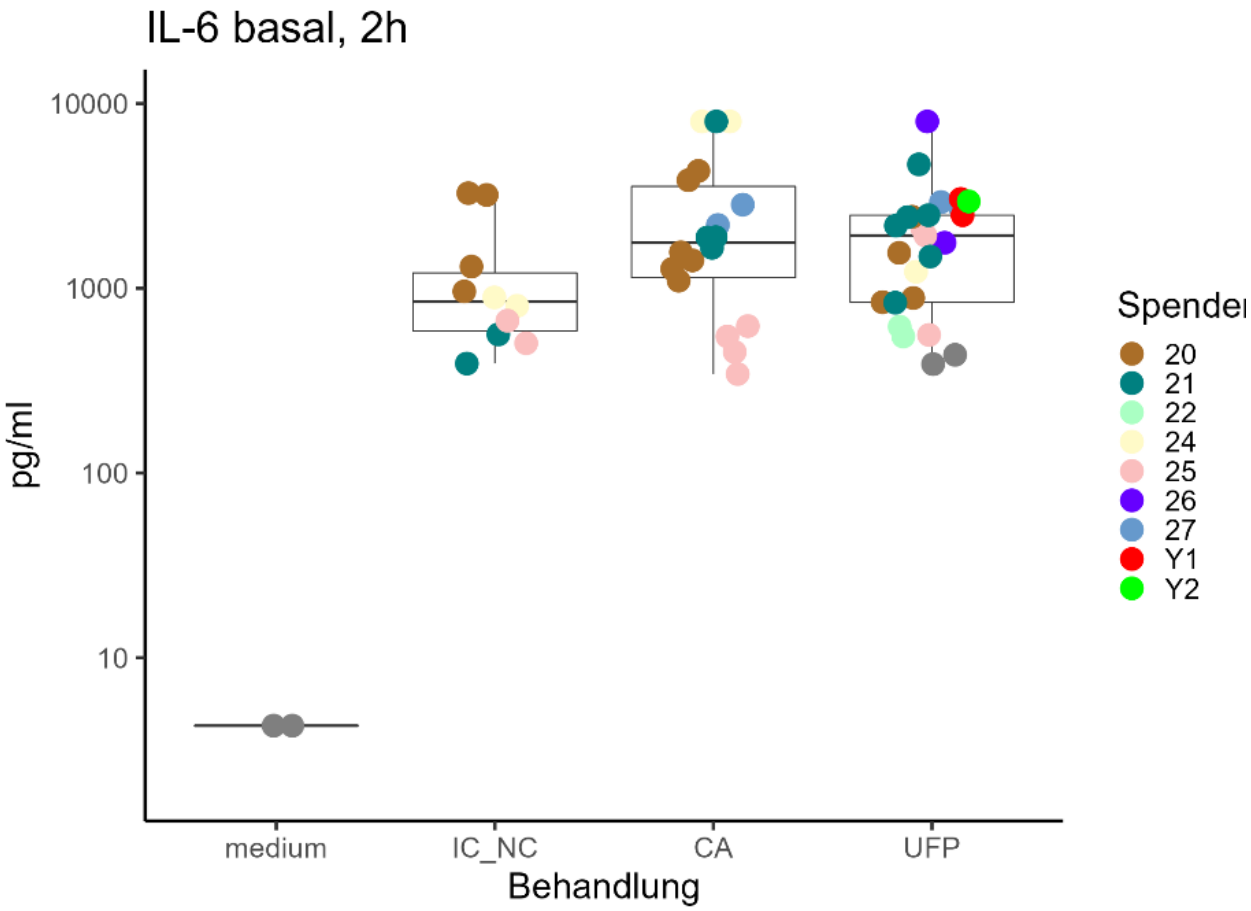
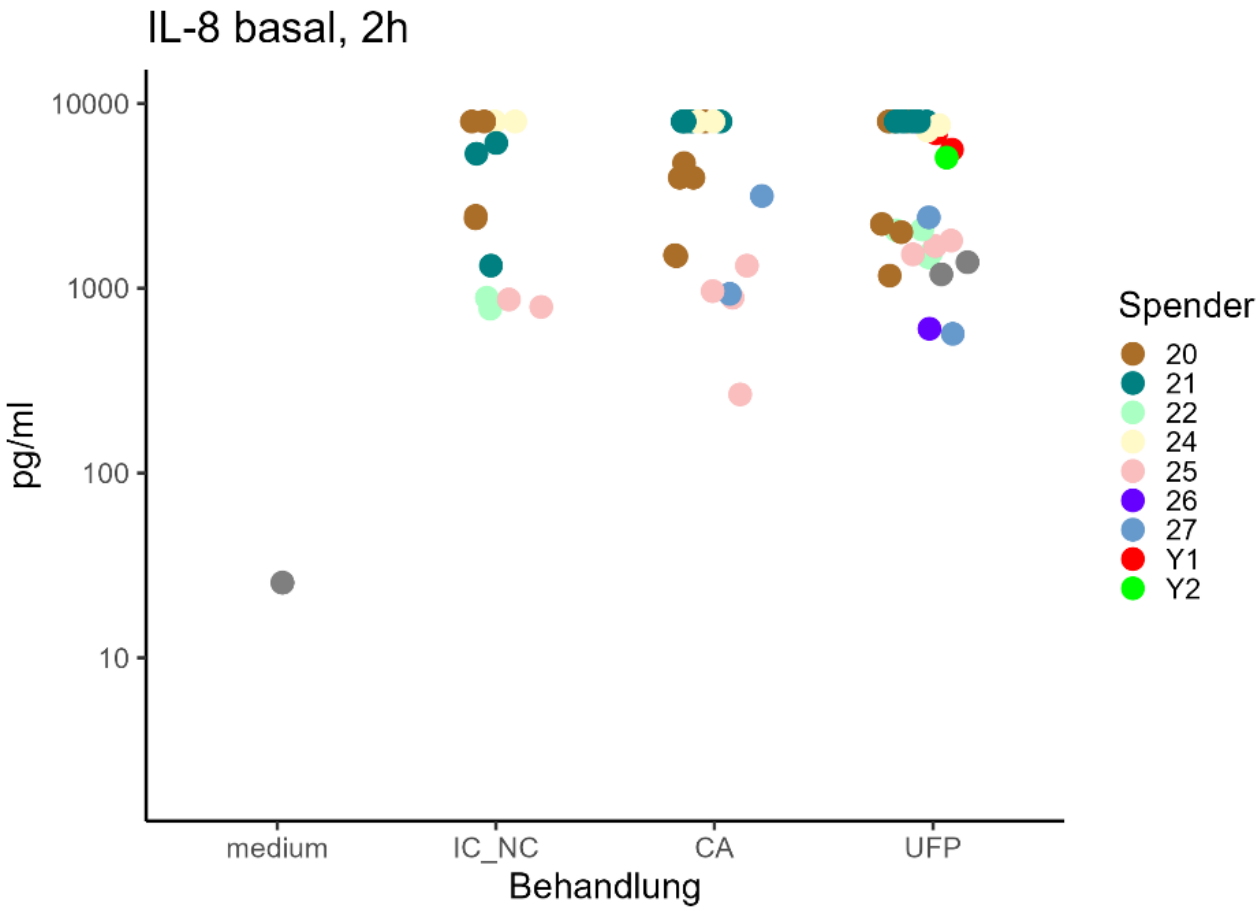
Cytokine release after UFP online exposition





Summary – UFP and respiratory mucosa models

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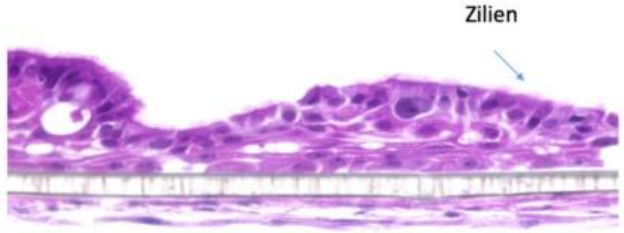
- Primary cell based respiratory mucosa models
  - High morphological *in vivo* – *in vitro* correlation
  - High functional relevance (ciliary beat, mucus)
  - Potential regeneration capacity
  - Long-term studies plausible
  - Established in complex exposure scenarios



Meyer, et. al.; 2023

- Effects of UFP on respiratory mucosa models
  - Minor effects on barrier and cell membrane integrity
  - No evidence for severe acute cytotoxicity or genotoxicity
  - In parts non-concentration dependend effects
  - Donor specific reactions

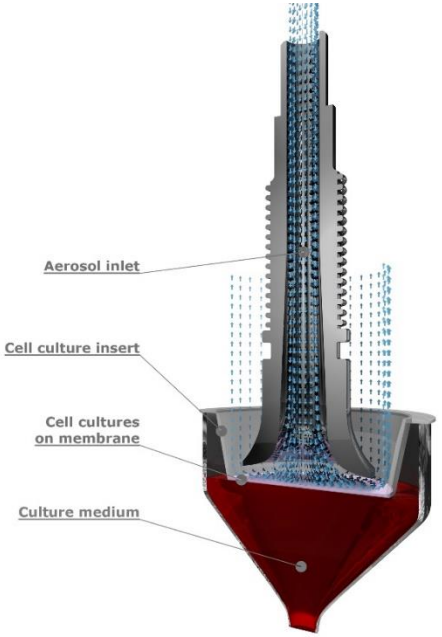


Image: Vitrocell (re-used with permission)

Thank you!

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