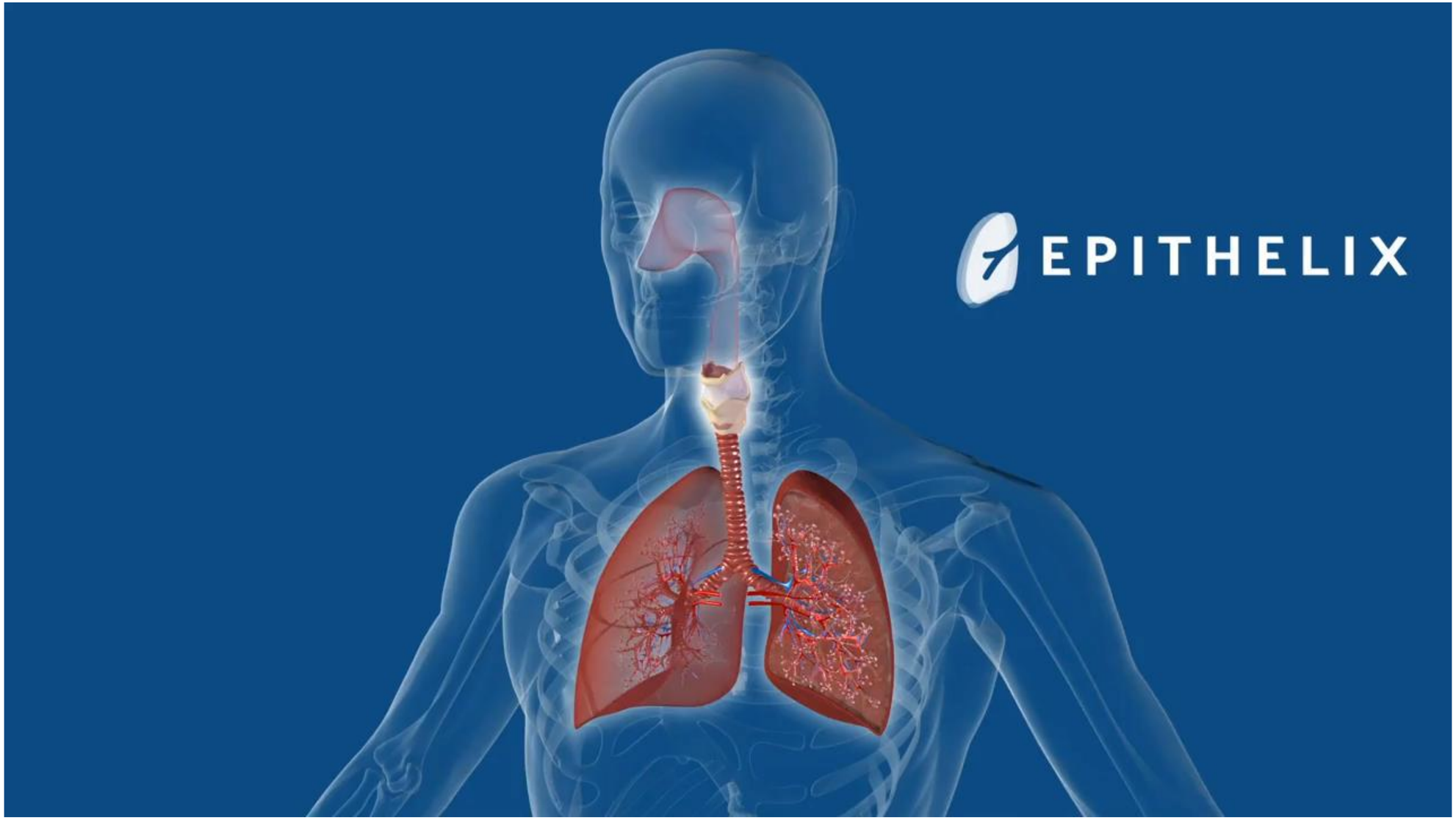
EPITHELIX
IN VITRO RESPIRATORY SOLUTIONS

Advanced immunocompetent in vitro primary human lung models for toxicity evaluation of airborne fibers and nanomaterials

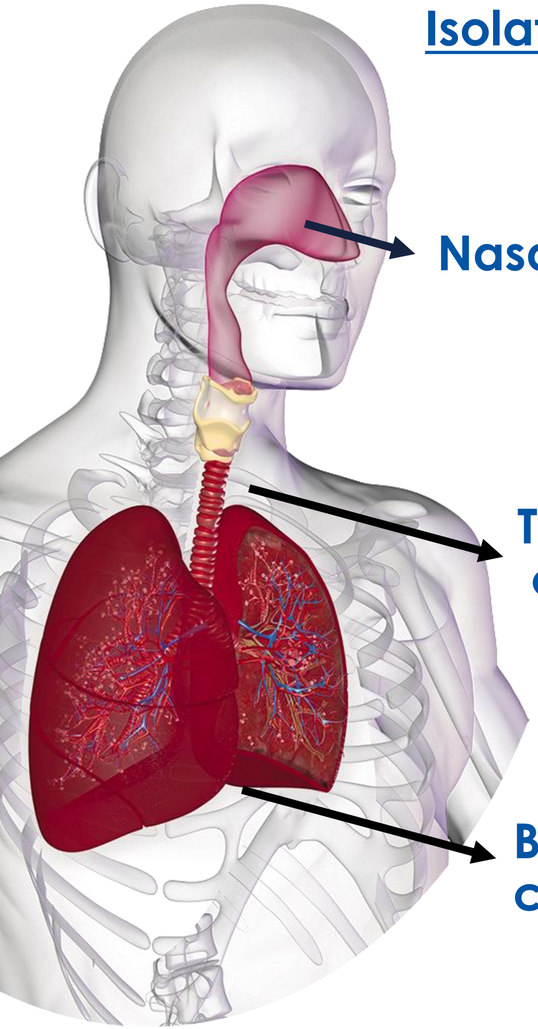
Samuel Constant, Ph.D., CEO
samuel.constant@epithelix.com

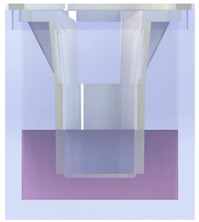
Adebiotech, Romainville, 04/12/2024

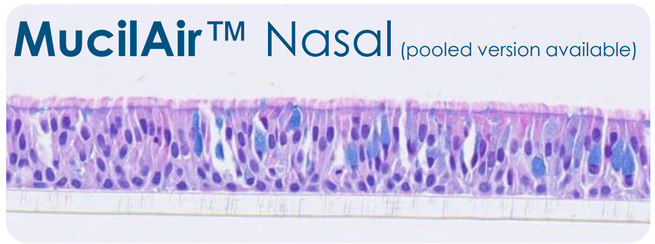
3D ALI airway epithelia products | MucilAir™ & SmallAir™

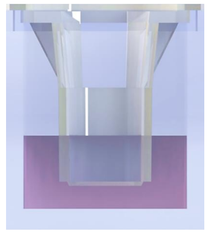


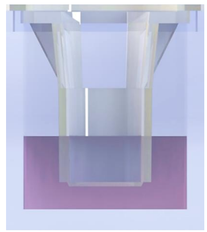
Isolation → **Cryopreservation** → **Seeding** → **ALI & differentiation**



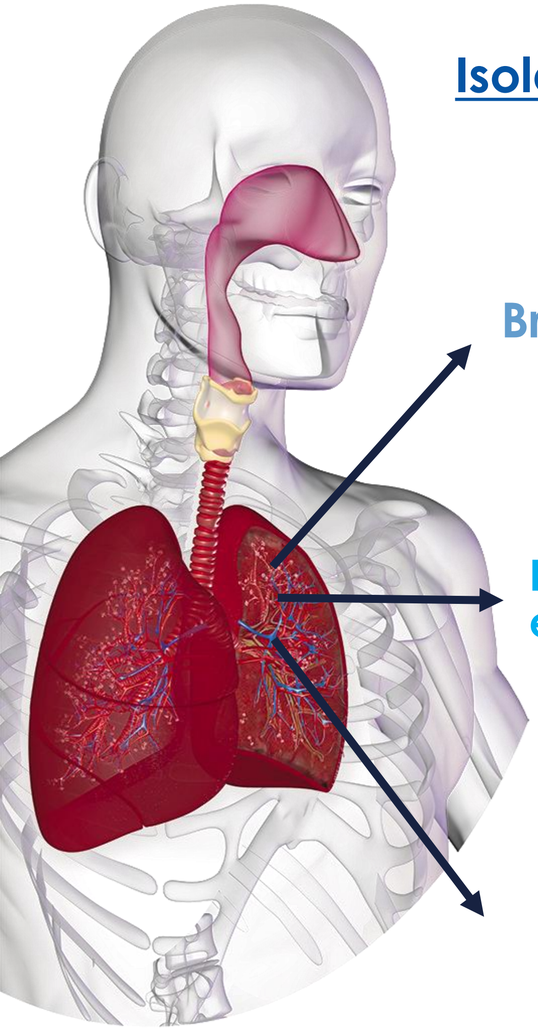
Nasal cells →  →  → 6 weeks → **MucilAir™ Nasal** (pooled version available)



Tracheal cells →  →  → 6 weeks → **MucilAir™ Tracheal**

Bronchial cells →  →  → 6 weeks → **MucilAir™ Bronchial**

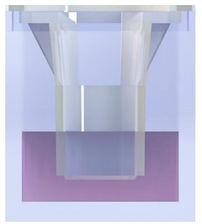
Reconstitution process SmallAir™ | AlveolAir™ Macrophages



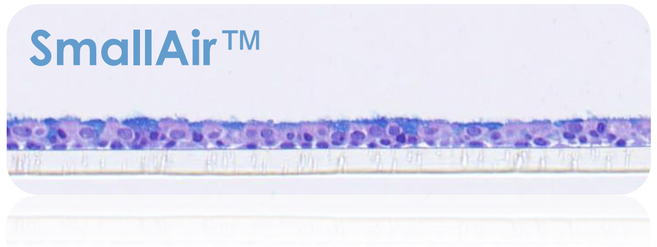
Isolation → **Cryopreservation** → **Seeding** → **ALI & differentiation**

Bronchiolar cells →

→

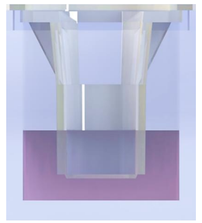


7 weeks →

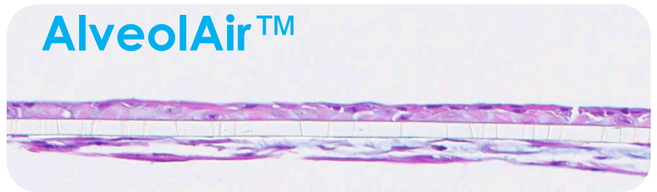


Pneumocytes & endothelial cells →

→



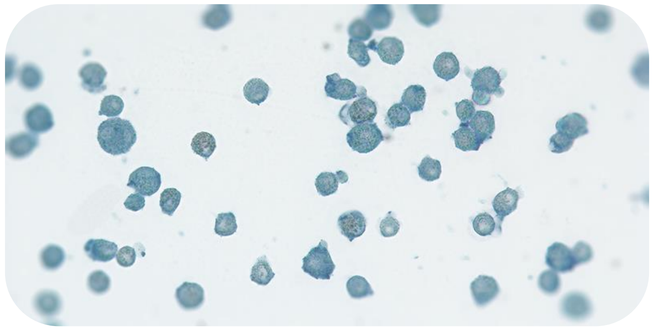
2 weeks →



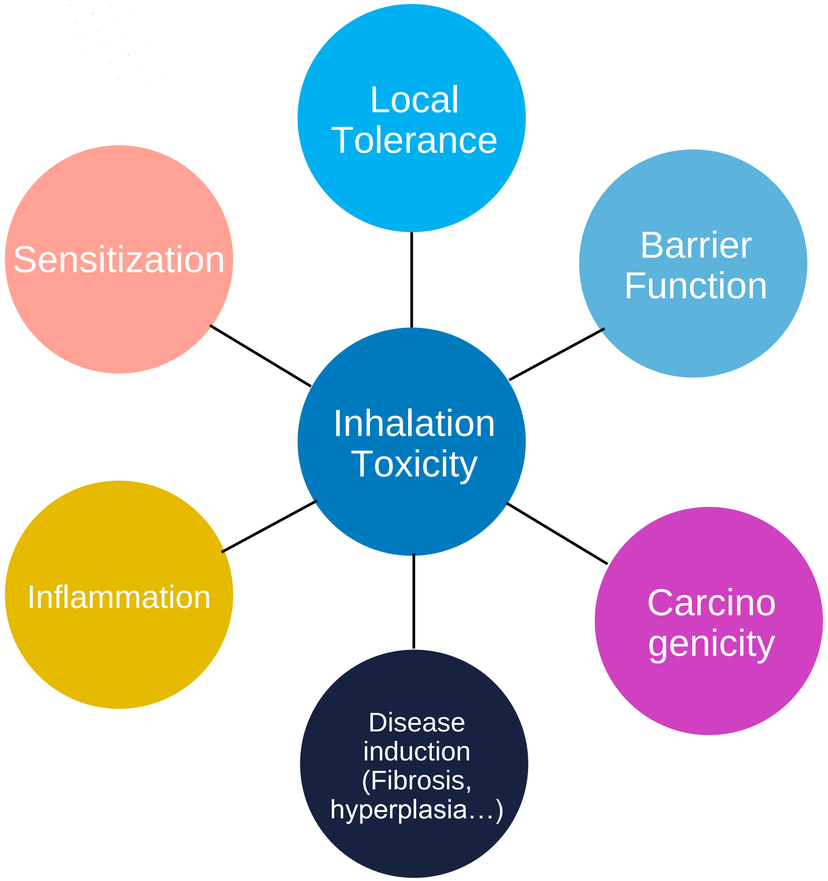
Isolation → **Cryopreservation**

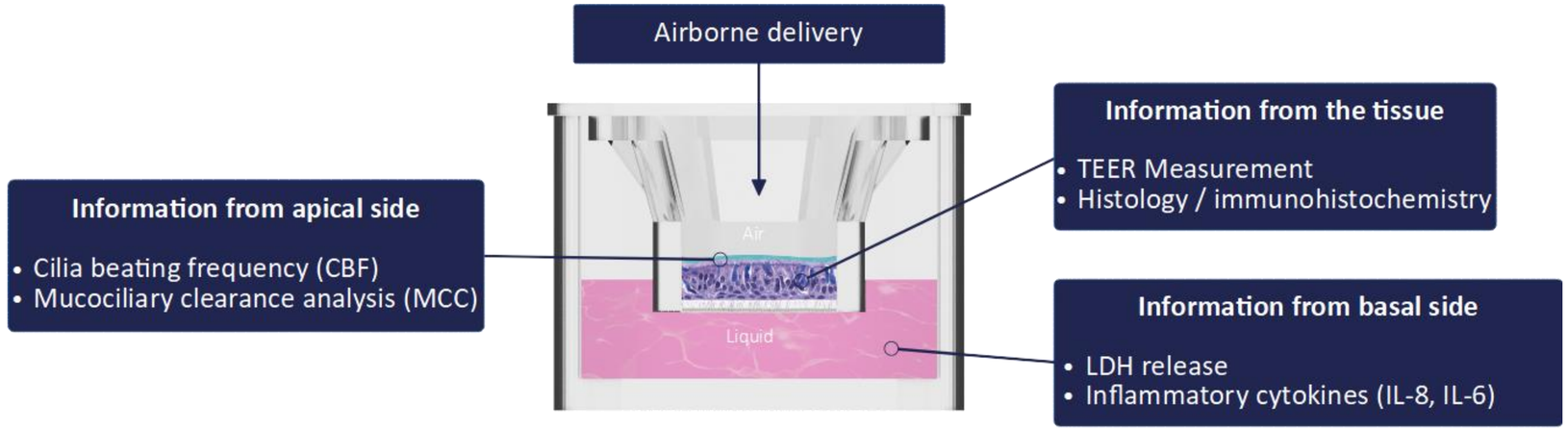
Alveolar Macrophages →

→

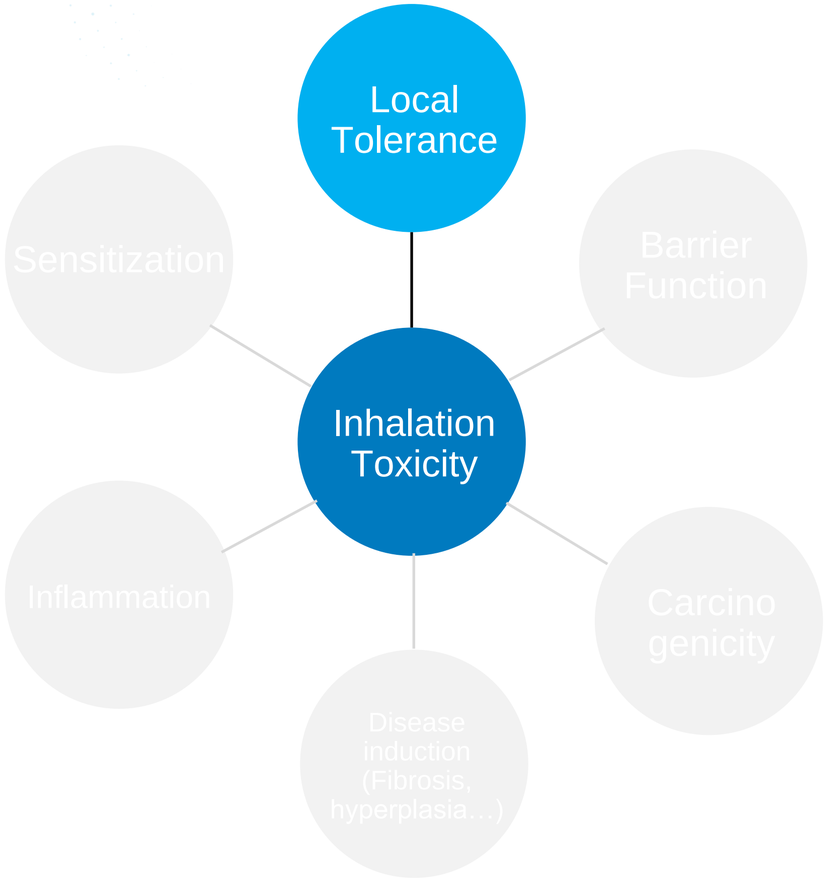


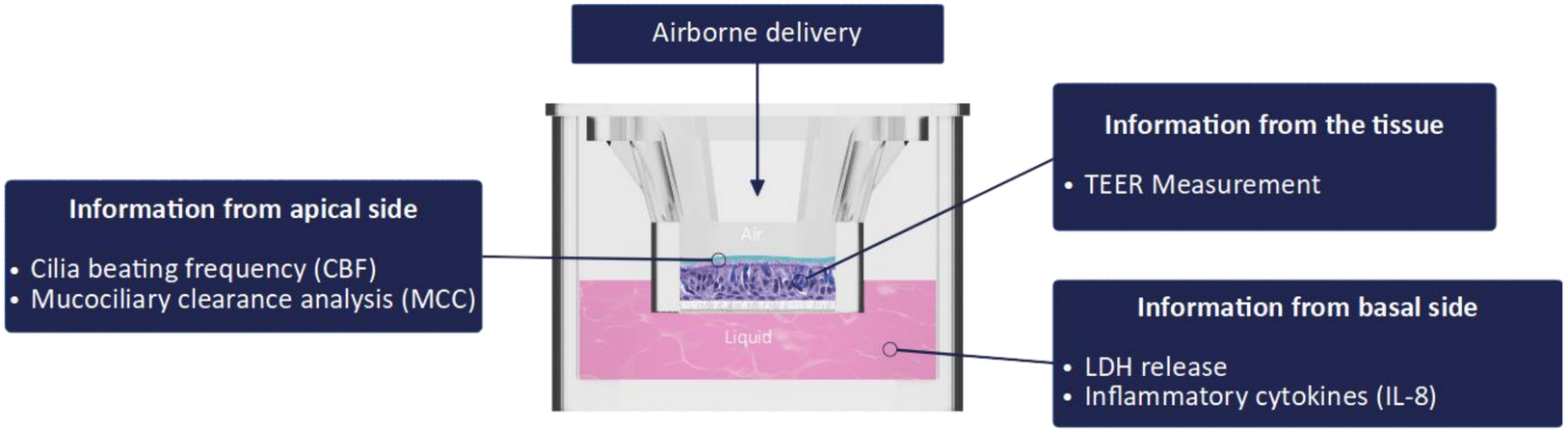
Evaluation of inhalation toxicity of inhaled xenobiotics/drugs





Evaluation of inhalation toxicity of inhaled xenobiotics/drugs

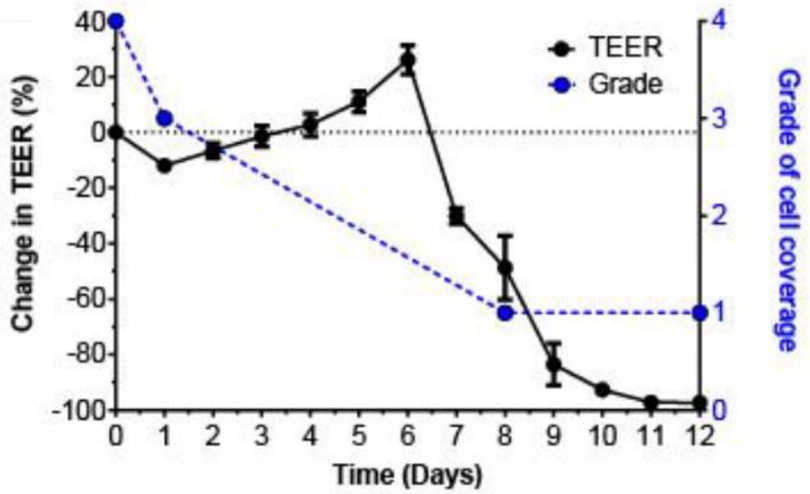


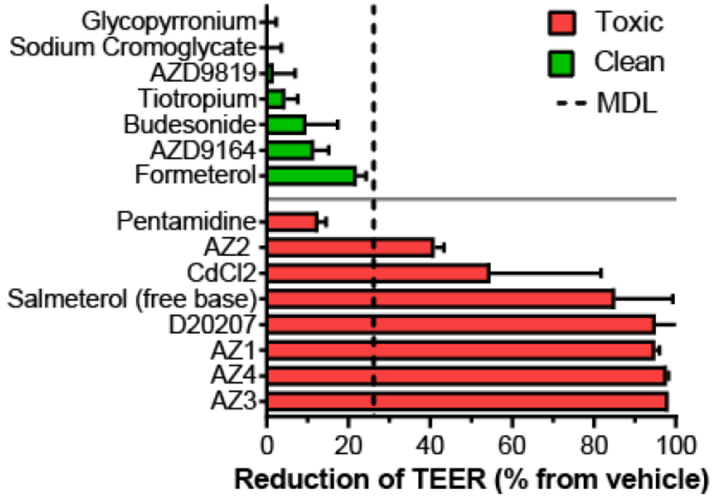


Prediction of respiratory Toxicity

A 3D human airway model enables prediction of respiratory toxicity of inhaled drugs *in vitro*

Kinga Balogh Sivars[1†]*, Ulf Sivars[4†], Ellinor Hornberg[4†], Hui Zhang[3†], Lena Brändén[3†], Rosy Bonfante[5], Song Huang[5], Samuel Constant[5], Ian Robinson[2††], Catherine J Betts[3††] and Per Åberg[2†]





15 compounds tested
88% sensitivity
100% specificity

Toxicol Sci. 2018 Mar 1;162(1):301-308.

Prediction of respiratory Toxicity using MucilAir™

Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2022)31

Unclassified

English - Or. English

1 September 2022

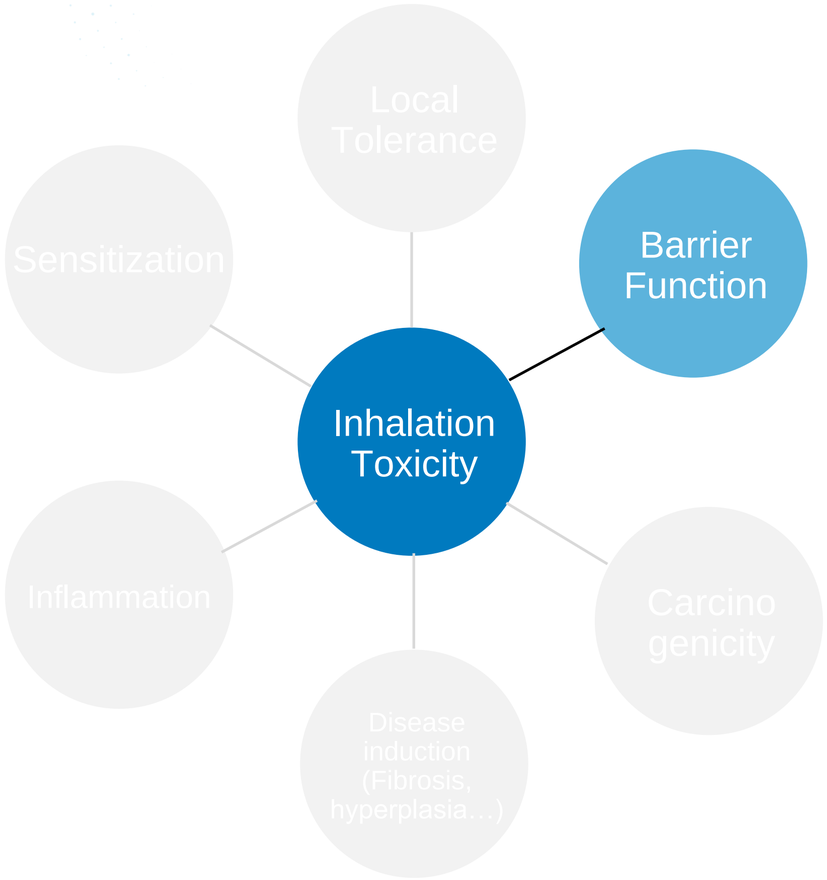
ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

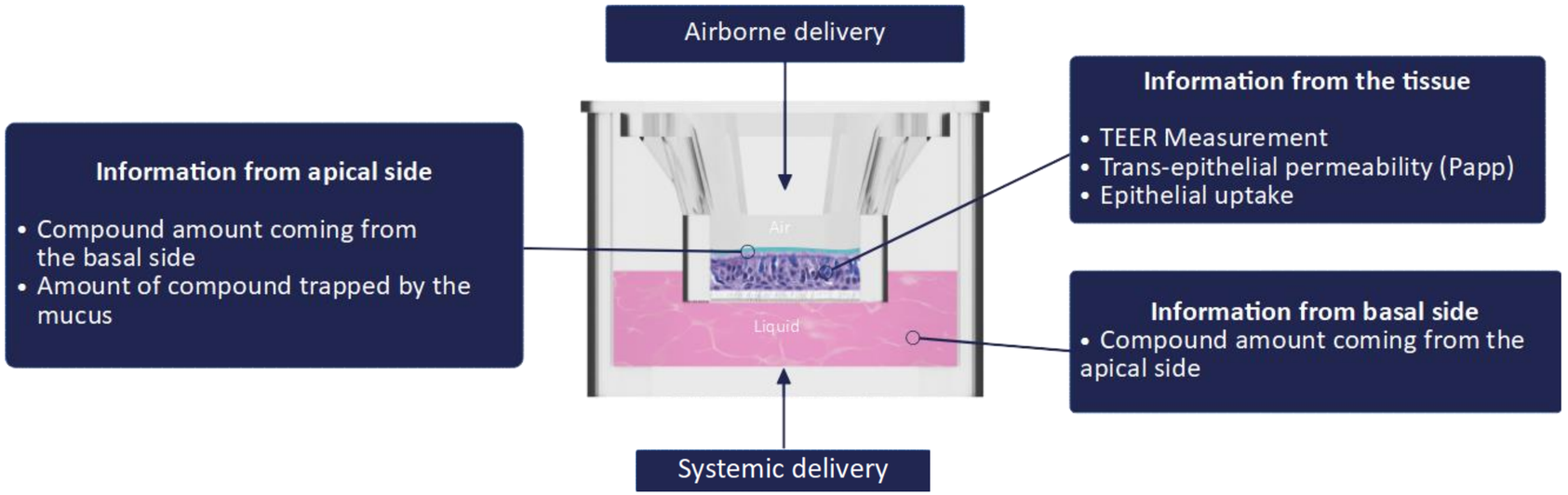
Case Study on the use of an Integrated Approach for Testing and Assessment (IATA) for New Approach Methodology (NAM) for Refining Inhalation Risk Assessment from Point of Contact Toxicity of the Pesticide, Chlorothalonil.

Series on Testing and Assessment
No. 367

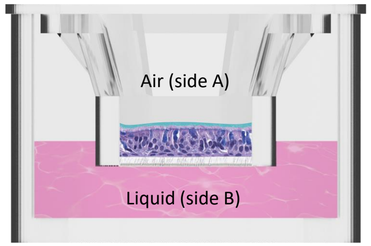
Replacement of a 90 Days rat inhalation study (OECD TG413) using MucilAir™

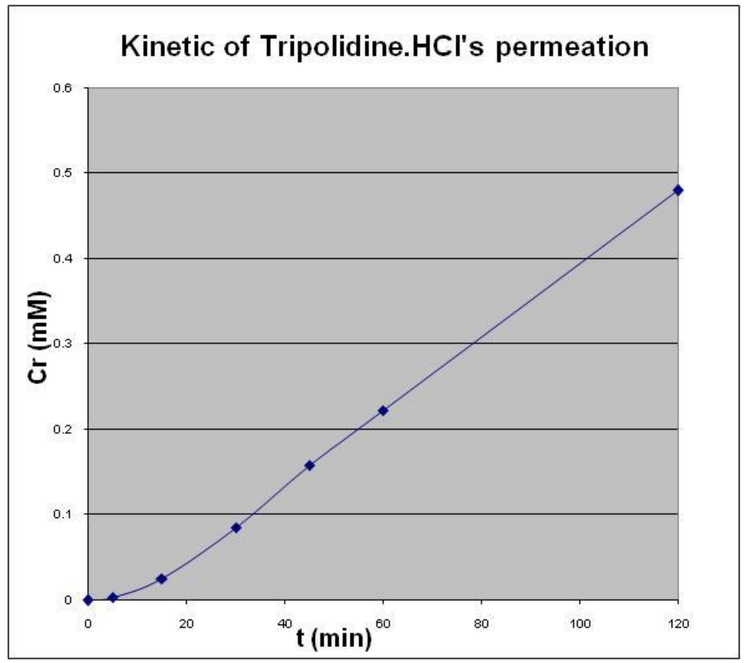
Respiratory Absorption of Drugs/Formulations or Xenobiotics





Respiratory Absorption of Drugs/Formulations





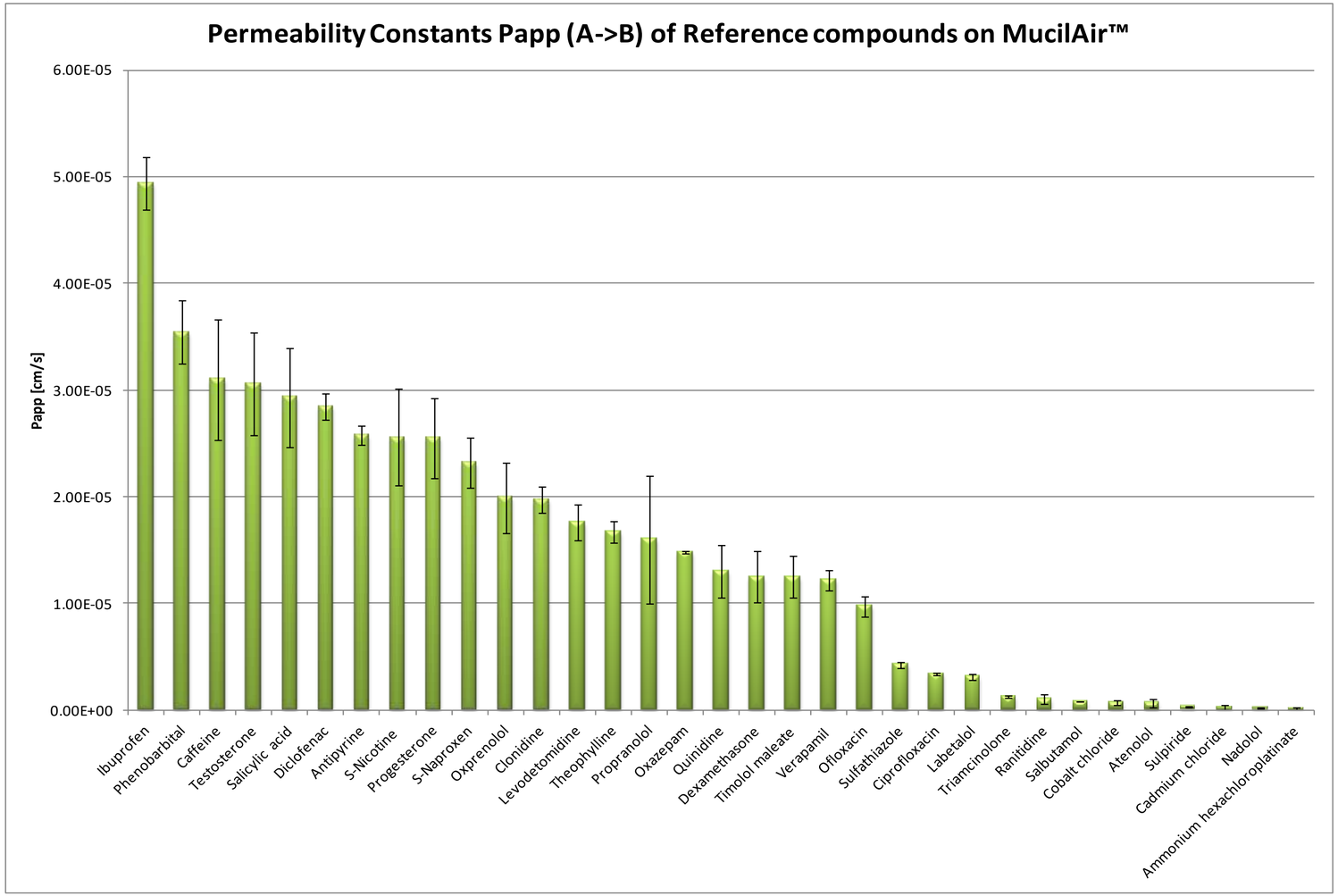
Time course of the rate of permeation of Tripolidine.HCl from the apical to basal lateral side (triplicate)

Molecules	Papp (cm/s) A→B	Papp (cm/s) B→A	Asymmetry Ratio
Salicilic Acid	7.7×10^{-5}	1.7×10^{-5}	0.2
Nicotine	2.1×10^{-5}	3.3×10^{-5}	1.6
Propranolol.HCl	1.2×10^{-5}	1.6×10^{-5}	1.3
Ibuprofen	1.1×10^{-5}	1.9×10^{-5}	1.7
Tripolidine.HCl	9.7×10^{-6}	1.2×10^{-5}	1.2
Tetracaïne.HCl	8.0×10^{-6}	1.1×10^{-5}	1.3
Dopamine.HCl	3.0×10^{-6}	2.5×10^{-6}	0.8
Atenolol	2.2×10^{-6}	6.7×10^{-6}	3.0

✓ LC-MS or ICP-MS detection

✓ High reproducibility

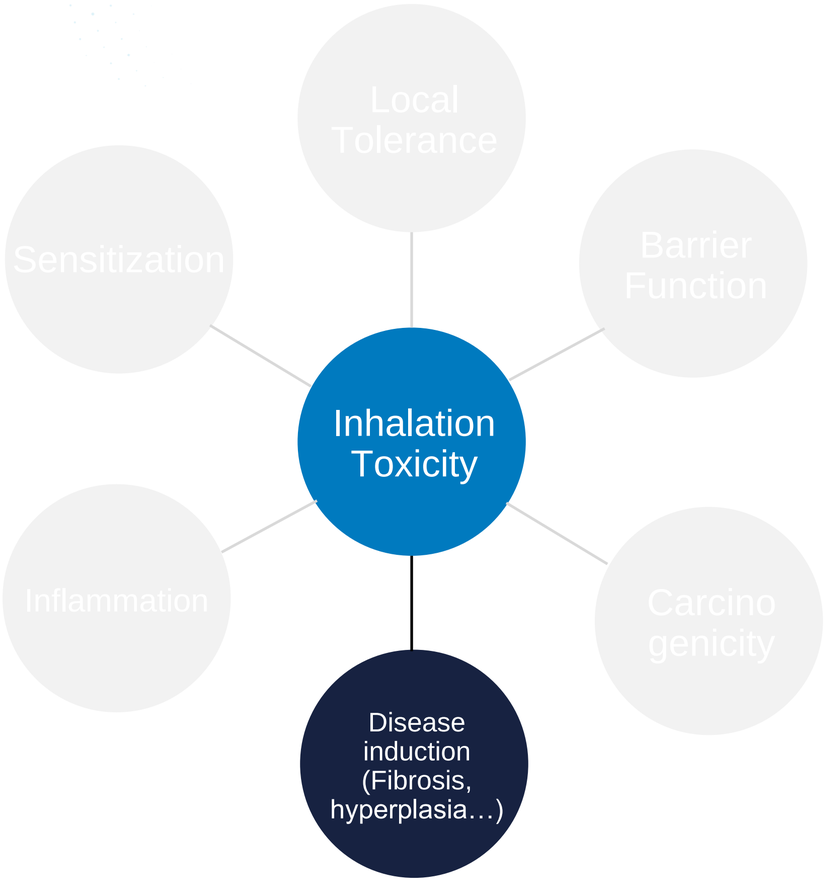
Respiratory Absorption of Drugs/Formulations

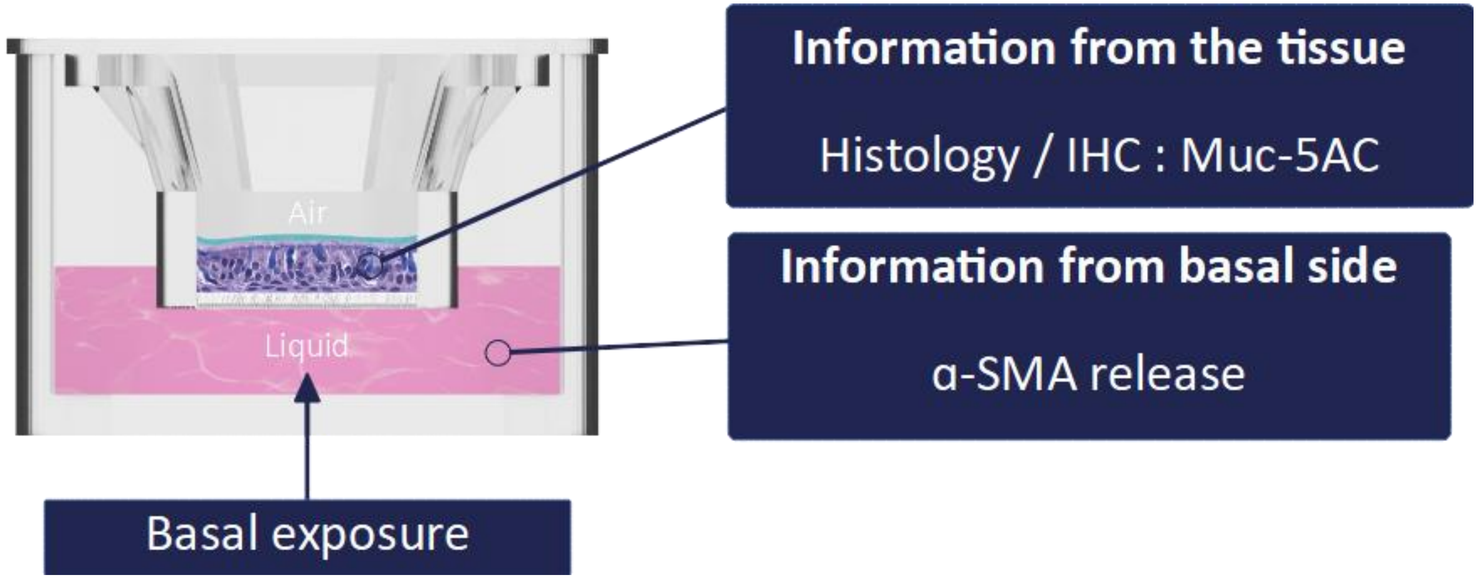


Apparent permeability coefficient (Papp) from A-B obtained from 33 reference compounds (n = 3). Data are expressed as mean – SD. SD, standard deviation.

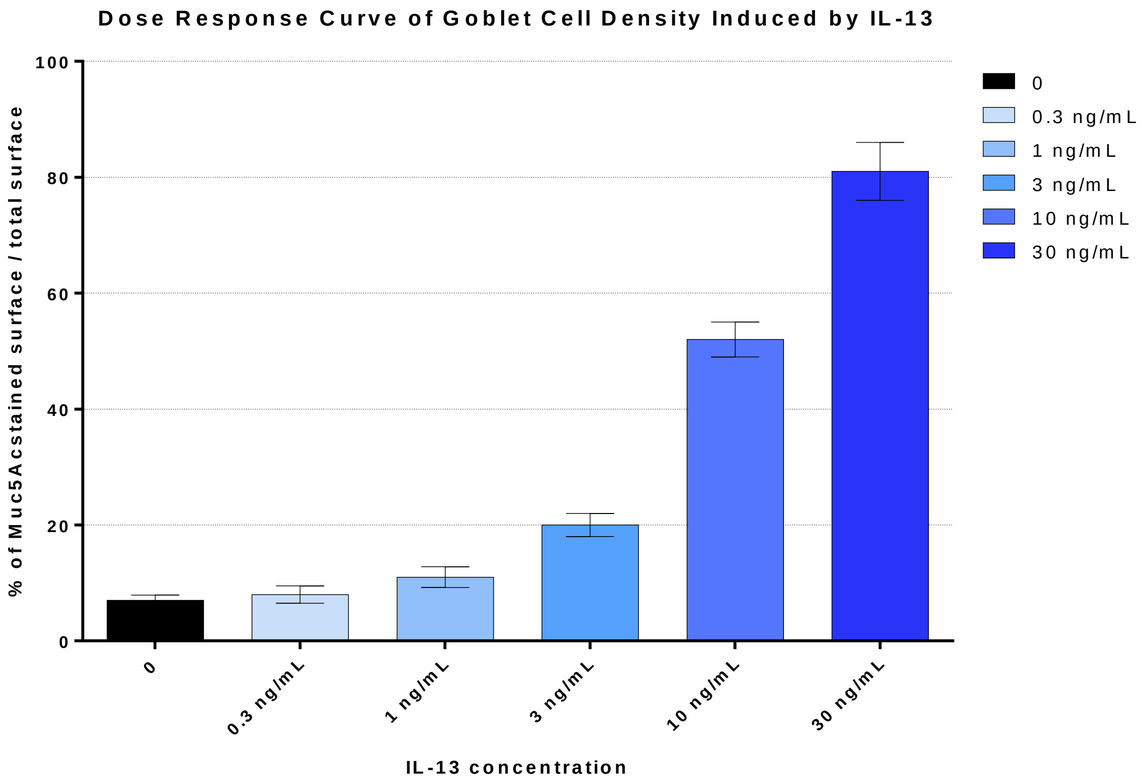
Hoffman et al. **Establishment of a Human 3D Tissue-Based Assay for Upper Respiratory Tract Absorption,** AIVT 2018

Respiratory Disease Induction





Goblet cell metaplasia detection



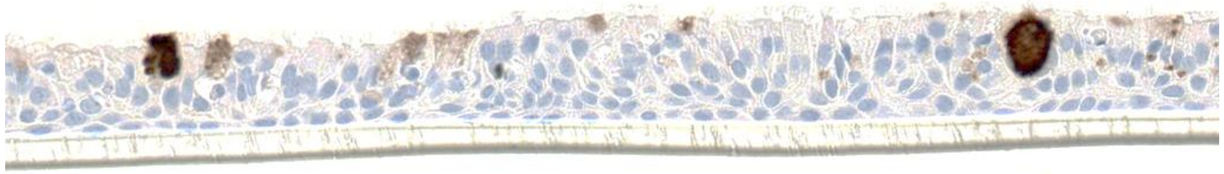


Figure 1: Control condition: 10% Goblet cells (staining with Muc5AC)

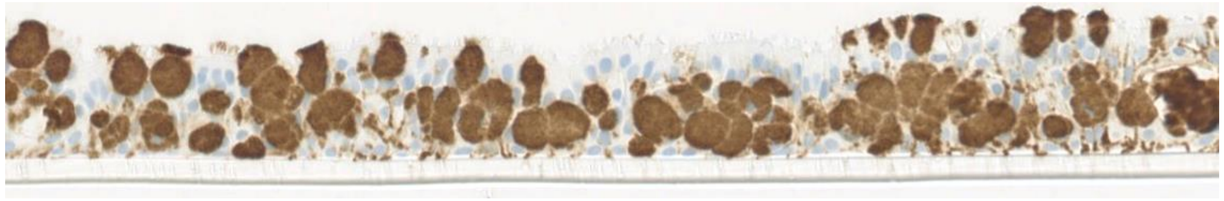
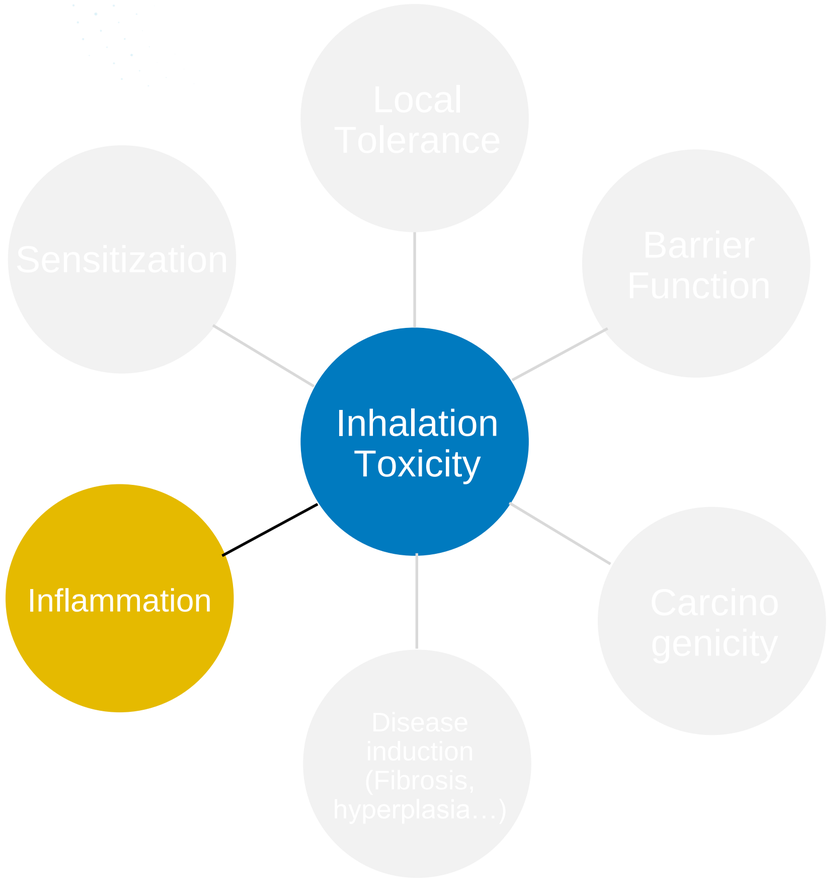
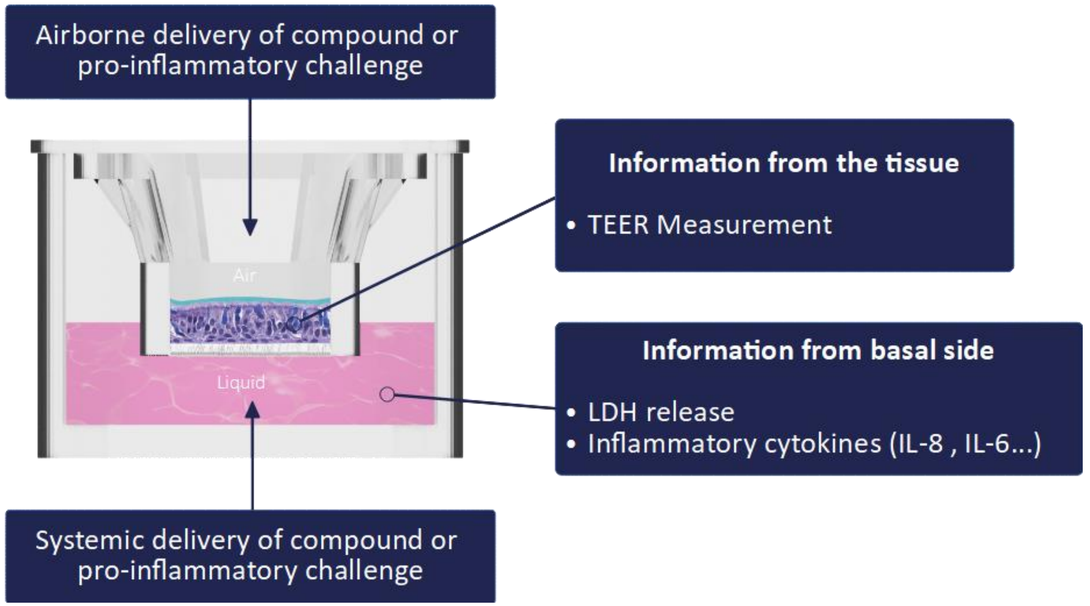


Figure 2: treatment with Il-13 (30 ng/ml) – 80% Goblet cells (staining with Muc5AC)

Evaluation of inhalation toxicity of inhaled xenobiotics/drugs

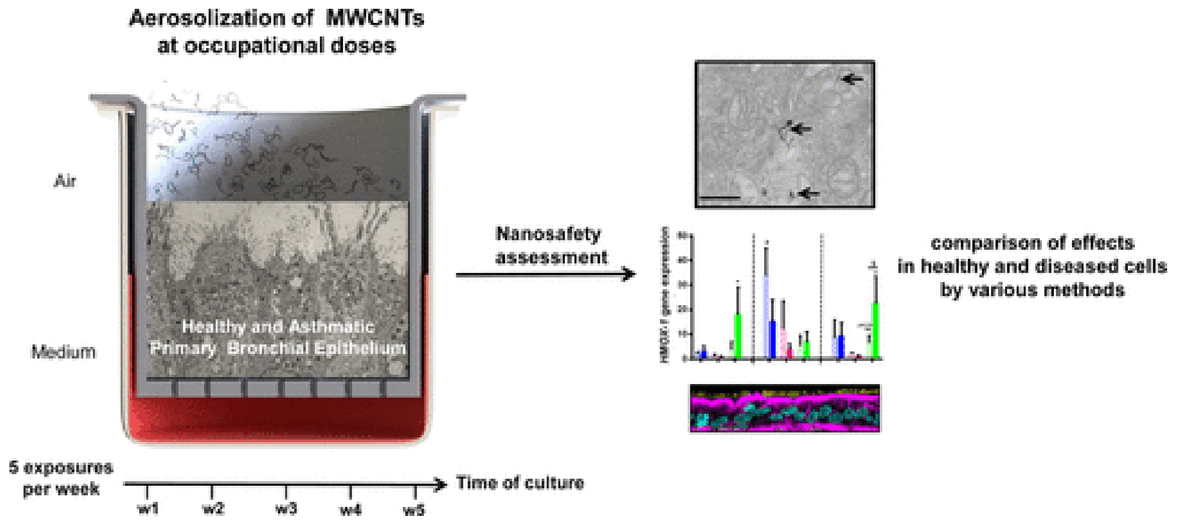




Pro-inflammatory monitoring

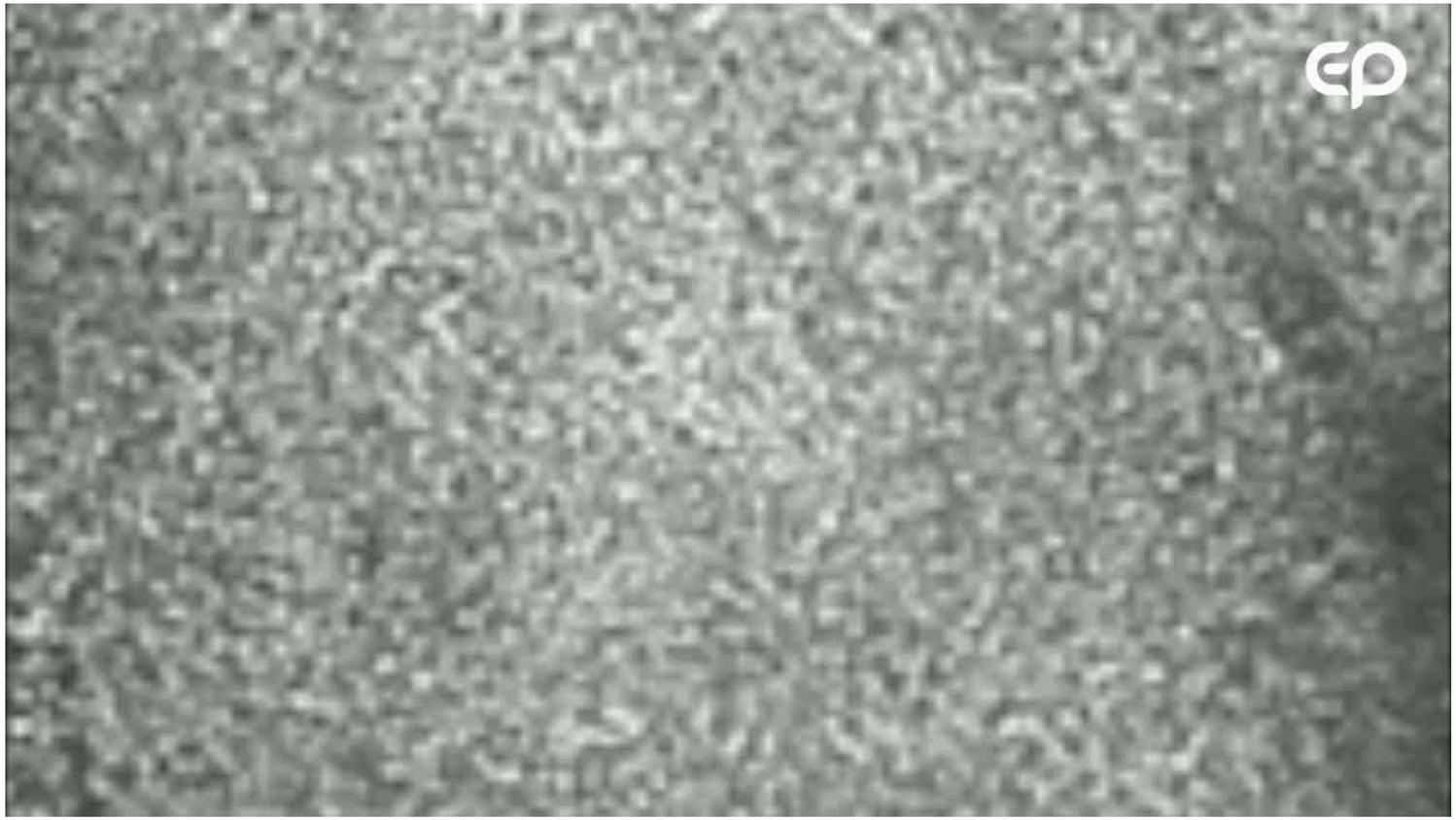
Human Asthmatic Bronchial Cells Are More Susceptible to Subchronic Repeated Exposures of Aerosolized Carbon Nanotubes At Occupationally Relevant Doses Than Healthy Cells

Savvina Chortarea,[†] Hana Barosova,[†] Martin James David Clift,[‡] Peter Wick,[§] Alke Petri-Fink,[†,#] and Barbara Rothen-Rutishauser[*,†]



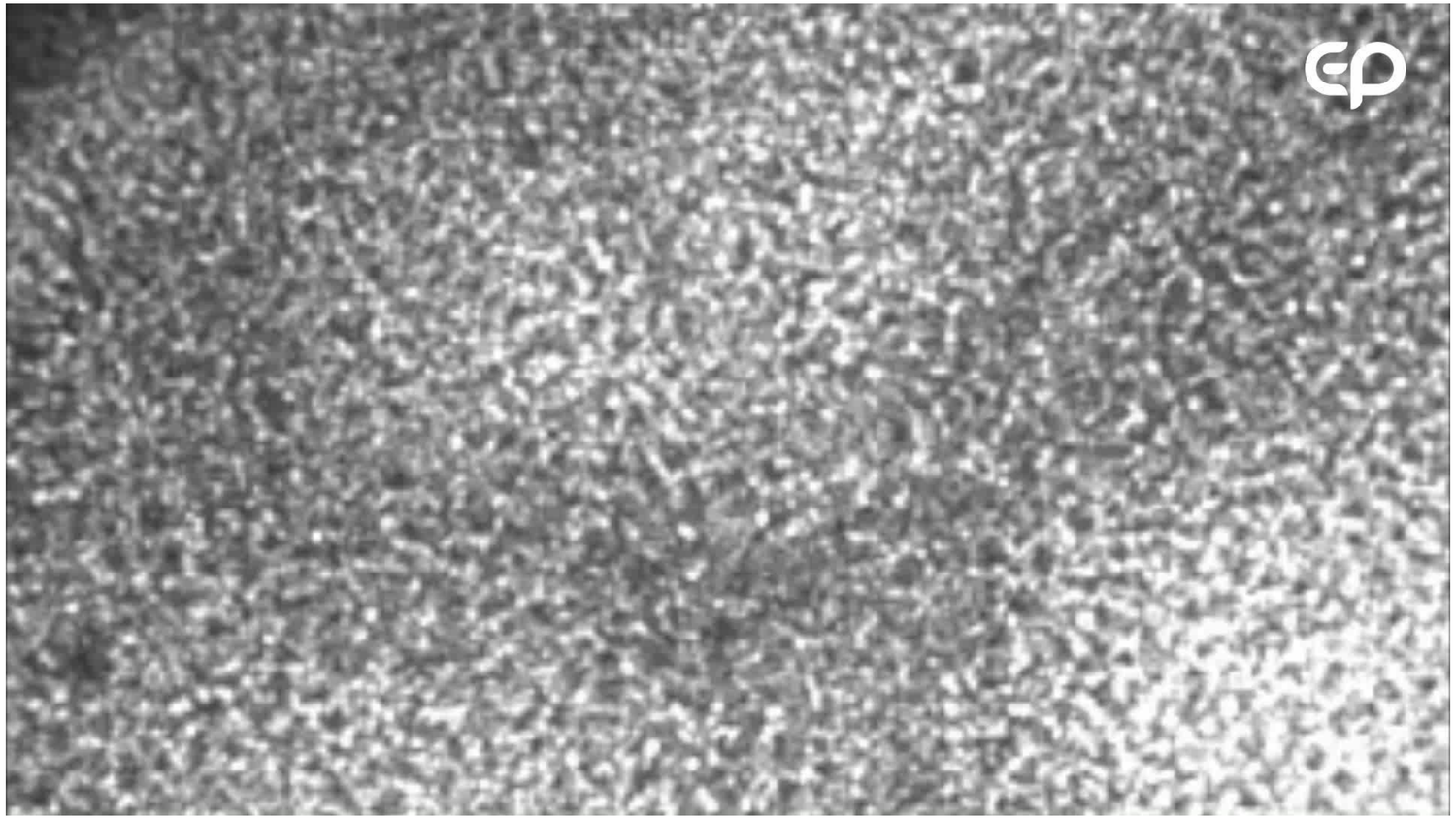
ACS Nano, 2017

Epithelix developed Cilia-X, a dedicated platform to measure cilia beating frequencies.



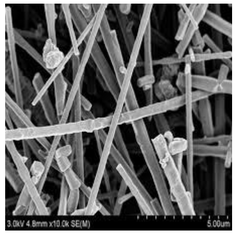
MucilAir™ healthy apical top view (phase contrast 5X, real time)

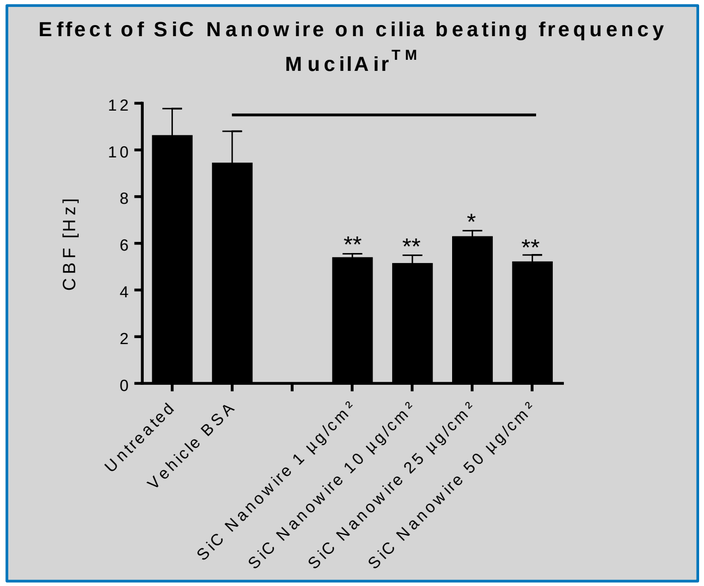
Mucociliary clearance (MCC) | analysis

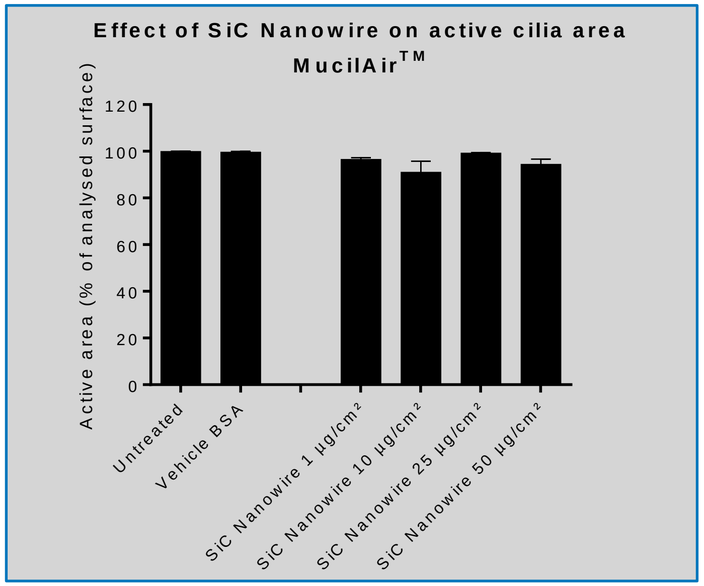


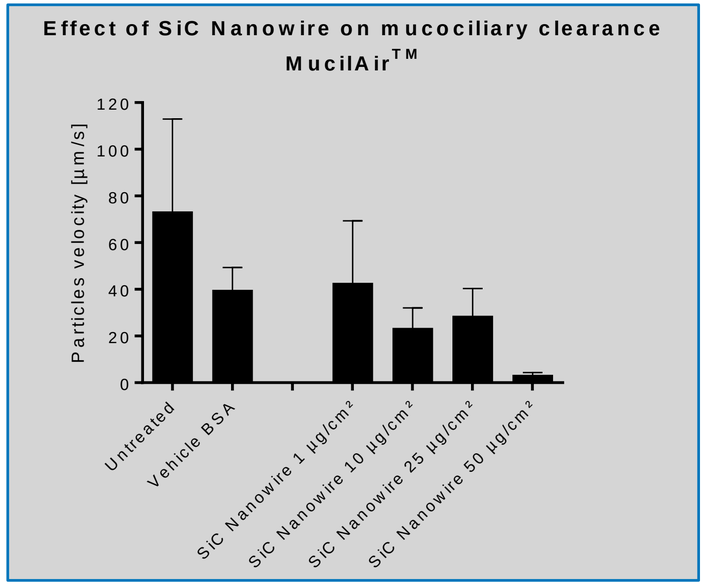
MucilAir™ healthy apical top view (phase contrast 5X, real time)

Ciliopathic evaluation of SiC nanowires



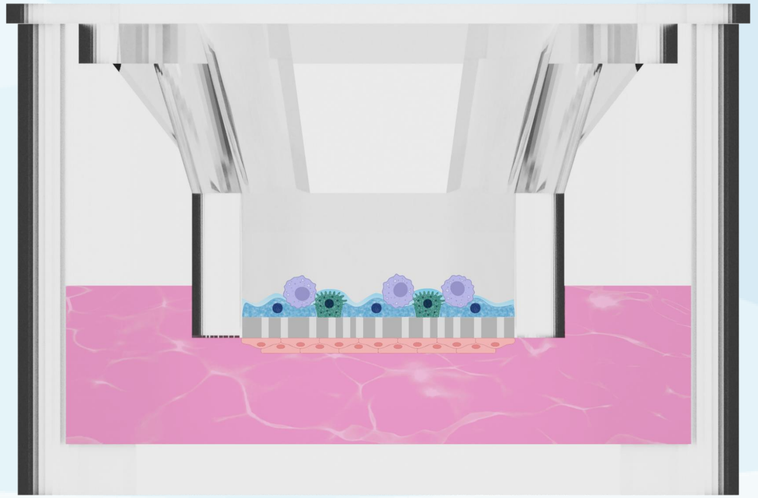




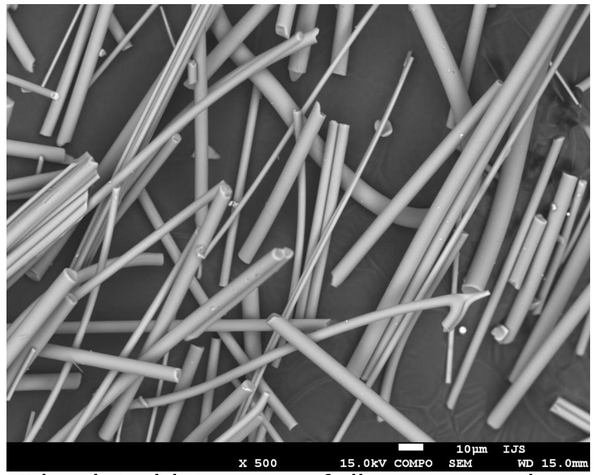


EPITHELIX
IN VITRO RESPIRATORY SOLUTIONS

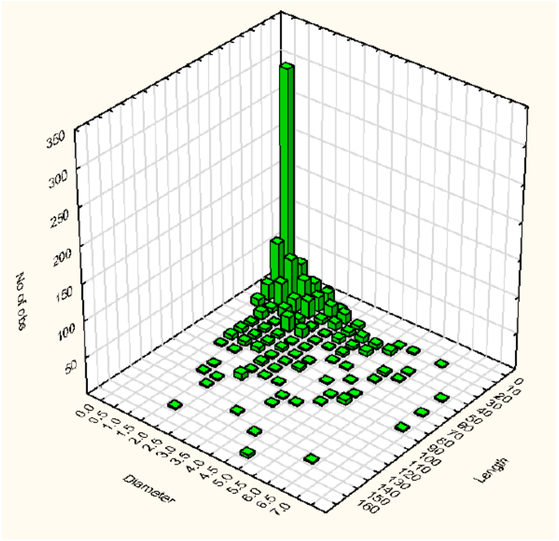
Phagocytosis of Man-Made Vitreous Fibers (MMVF) by primary human resident alveolar macrophages (AlveolAir-Macrophages)



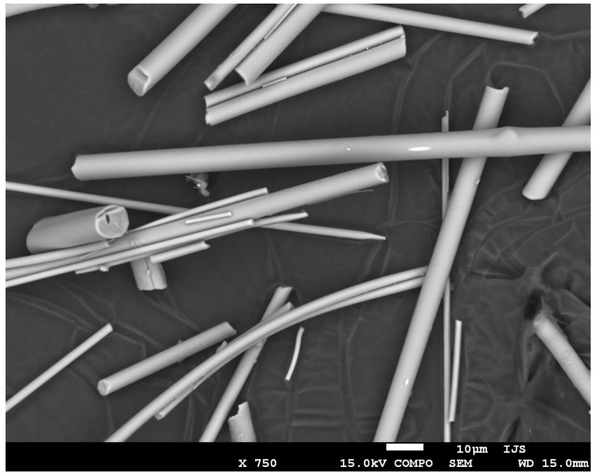
Glasswool



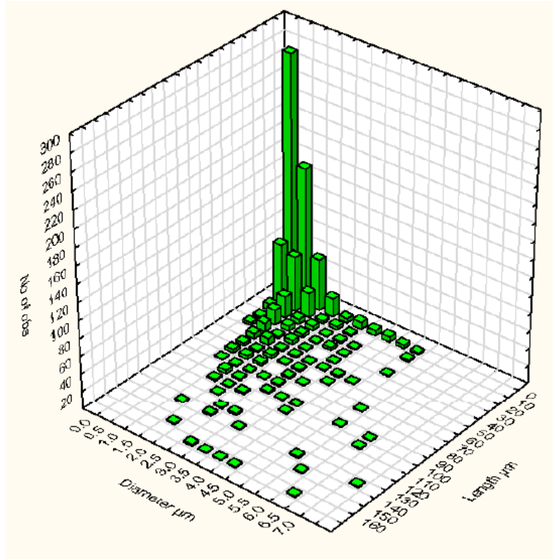
Bivariate histogram of diameter against length in Glass wool samples



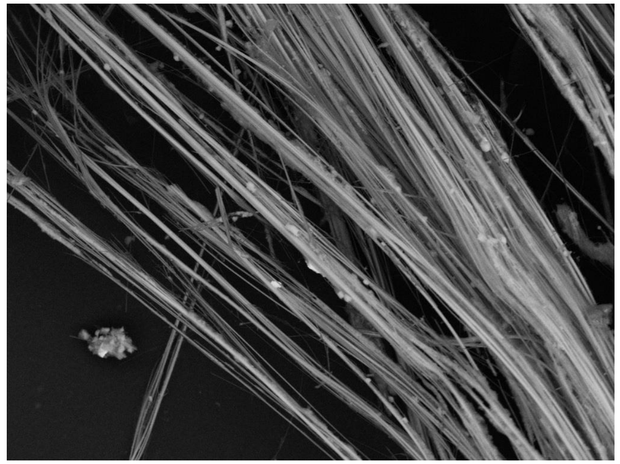
Rockwool



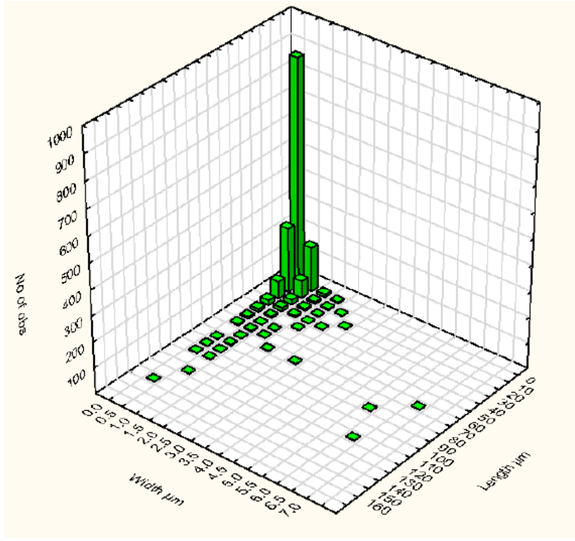
Bivariate histogram of diameter against length in rock wool samples



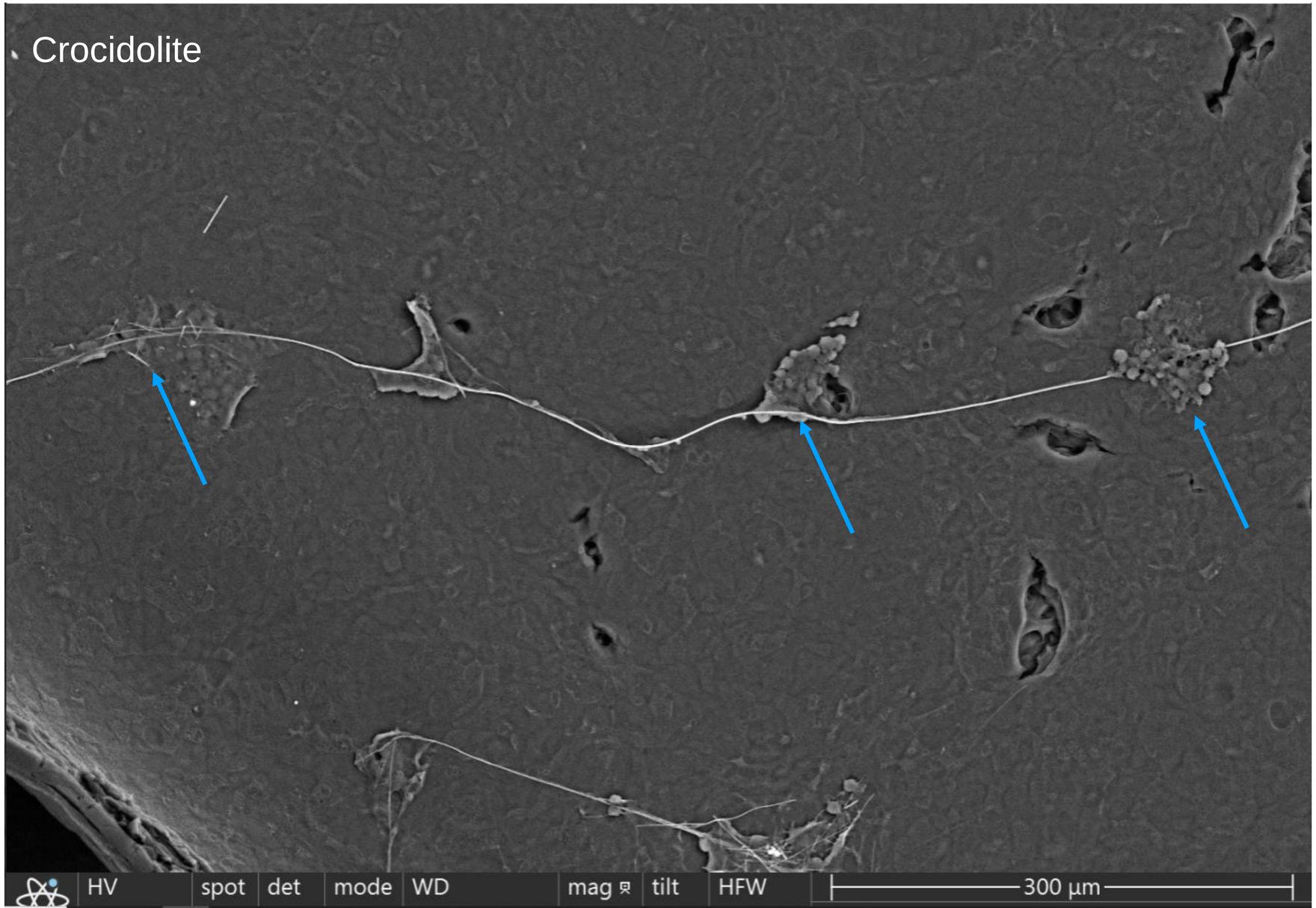
Crocidolite



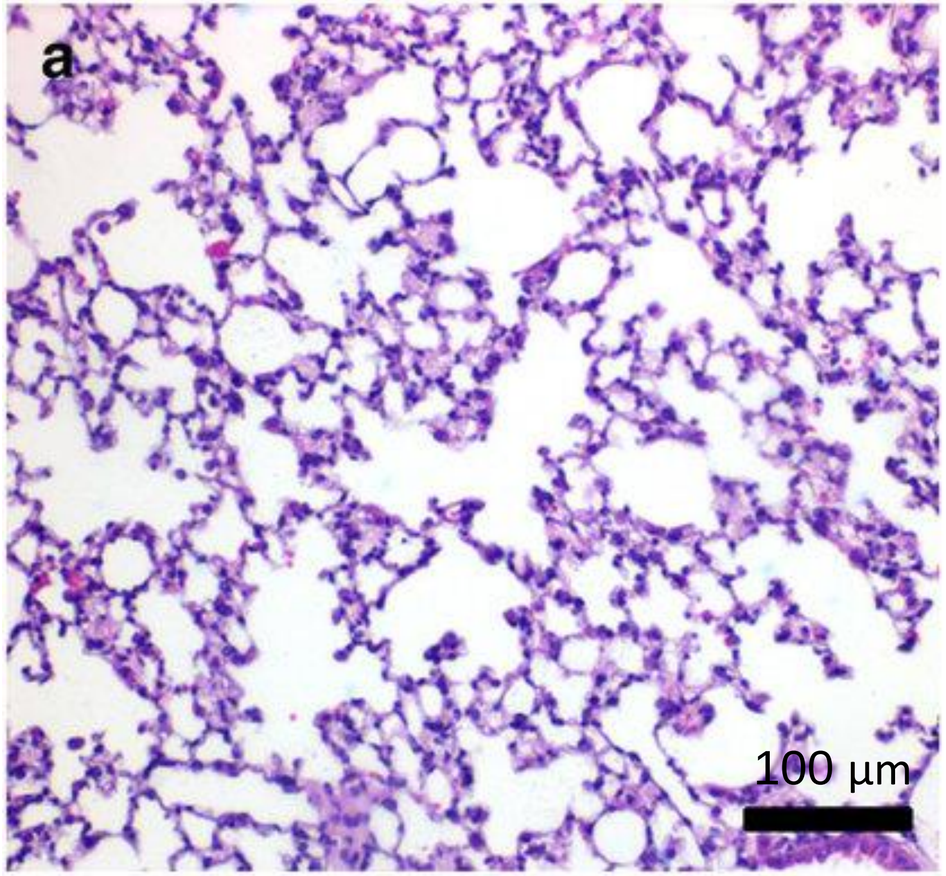
Bivariate histogram of diameter against length in crocidolite samples

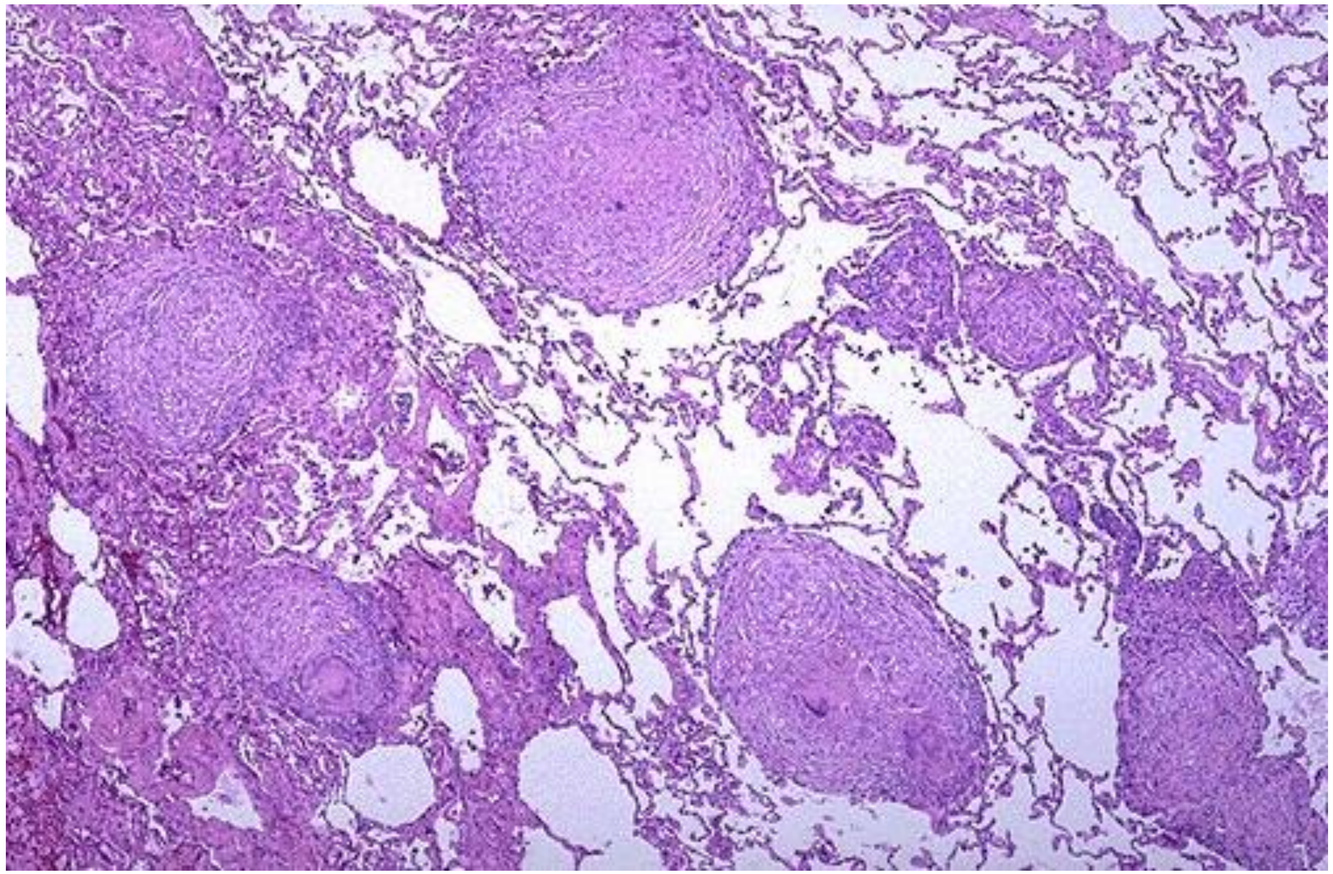


Chemotaxis of alveolar macrophages



Granuloma in lung parenchyma

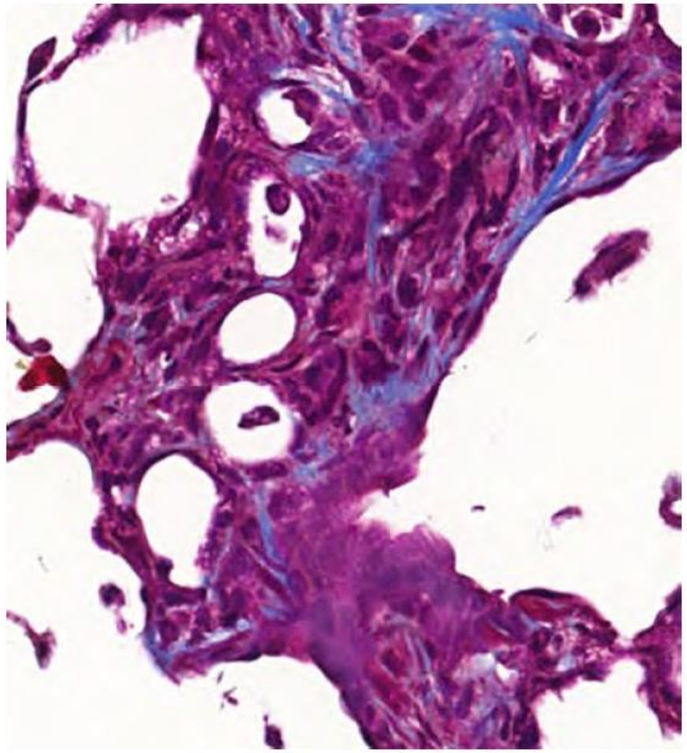


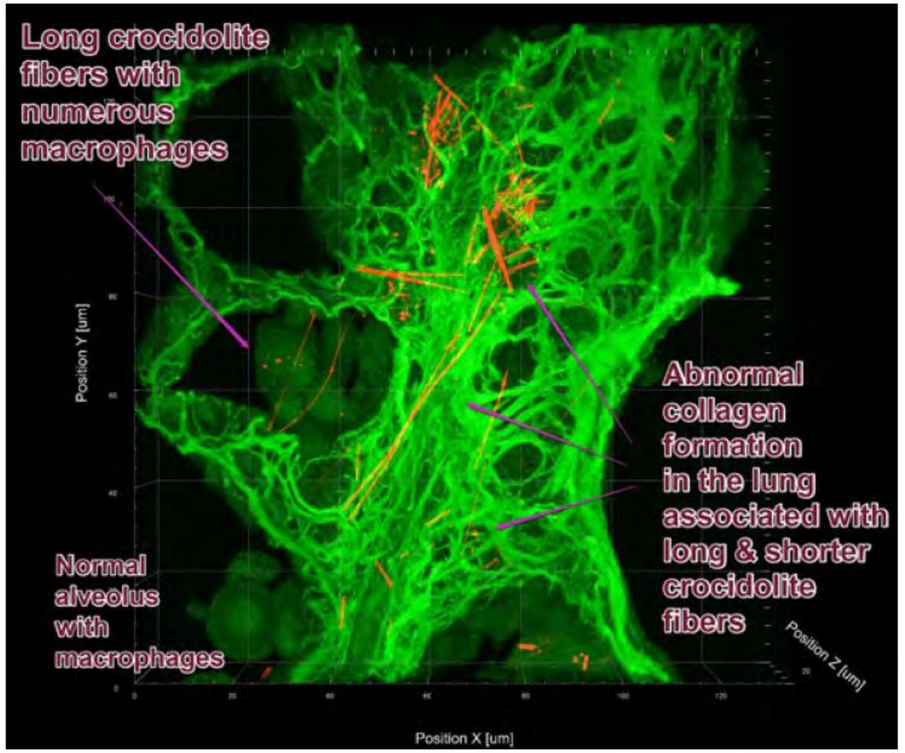


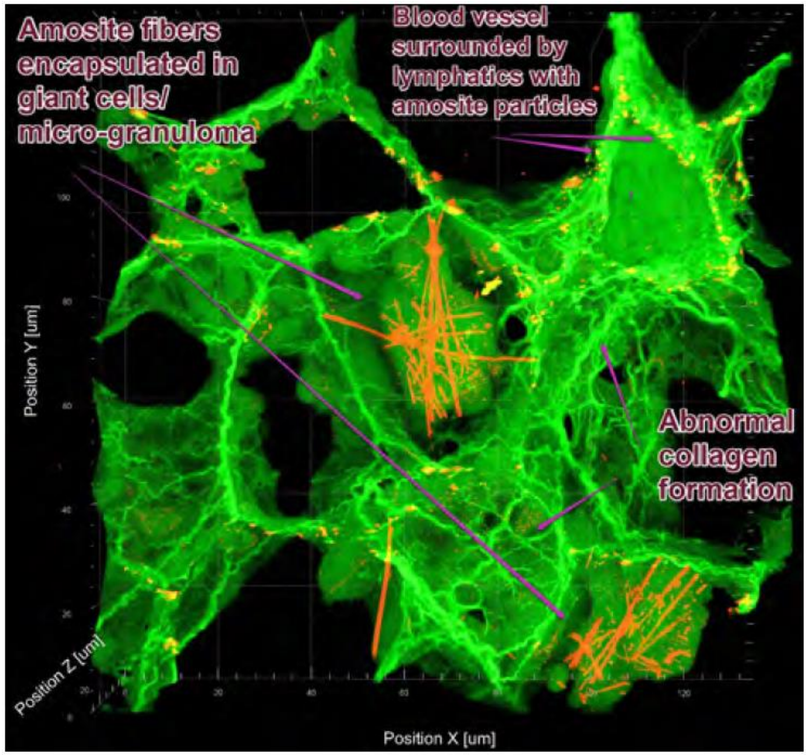
Granuloma: aggregate of macrophages in response to inflammation caused by unremoved foreign body. Activated macrophages can merge to form an “epithelioid” barrier[1].

1. https://www.ncbi.nlm.nih.gov/books/NBK554586/

Crocidolite induces Granuloma in rat lung parenchyma



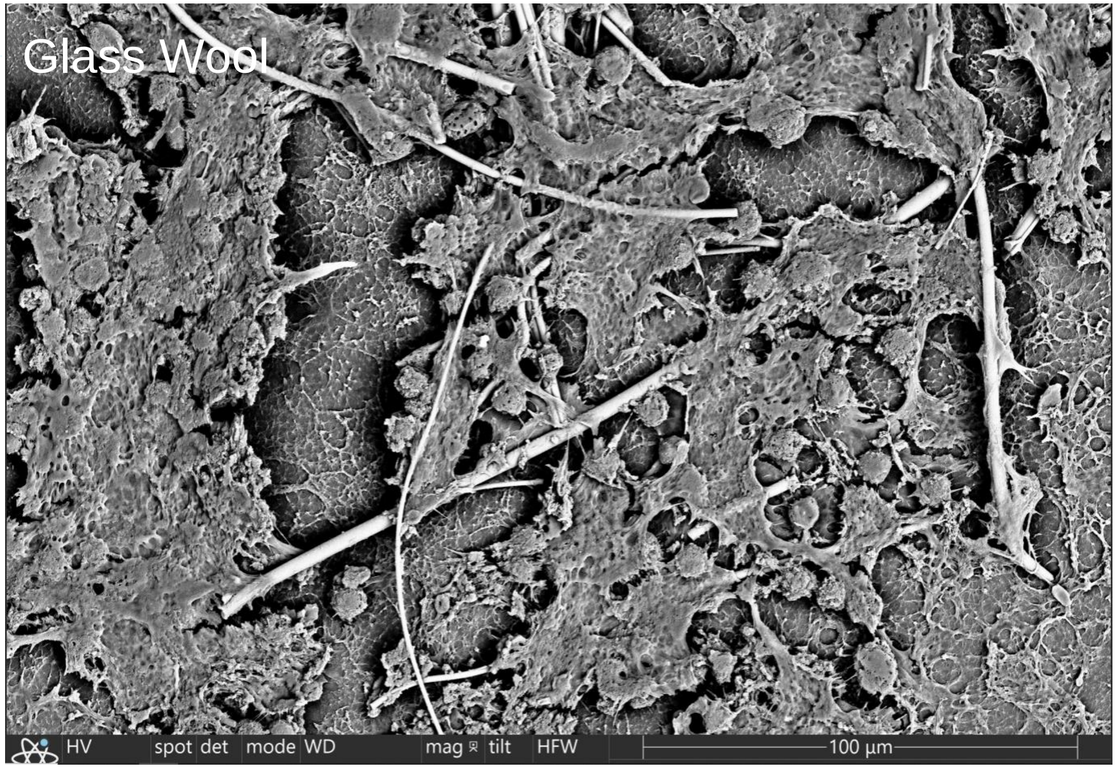


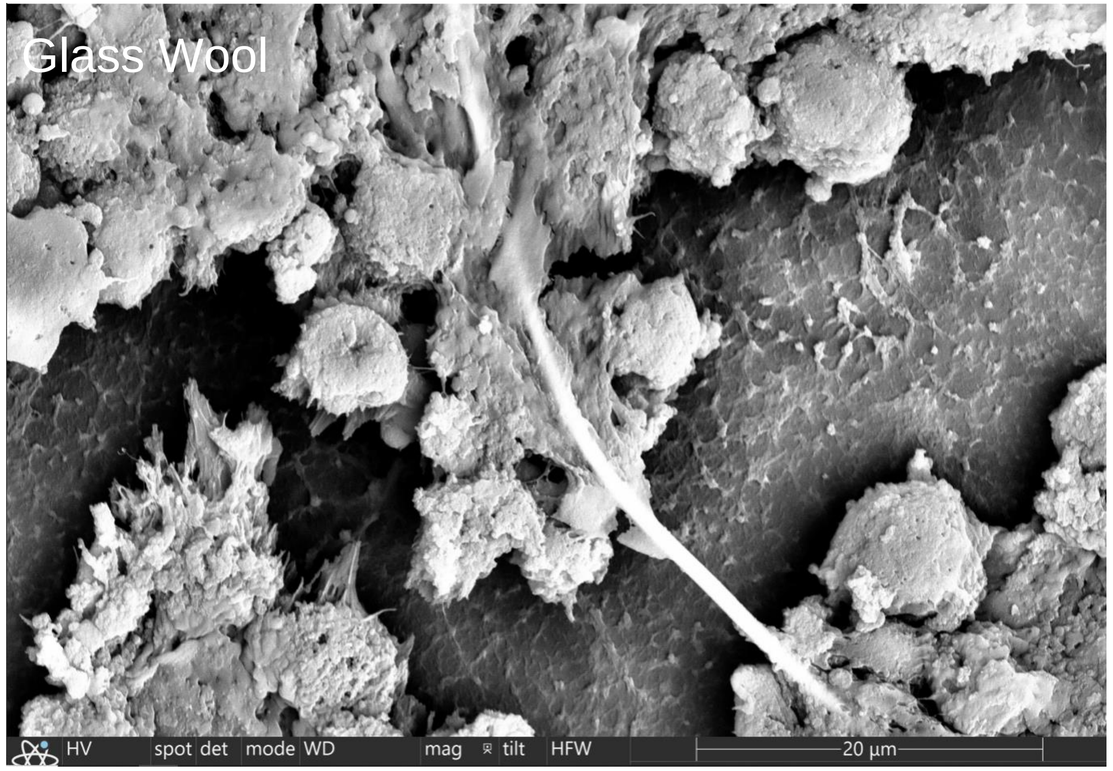


Granuloma: aggregate of macrophages in response to inflammation caused by unremoved foreign body. Activated macrophages can merge to form an “epithelioid” barrier[1].

1. Bernstein et al., Tox. And App. Pharm., 2019

Macrophages “granuloma” formation in AlveolAir™-Macrophages

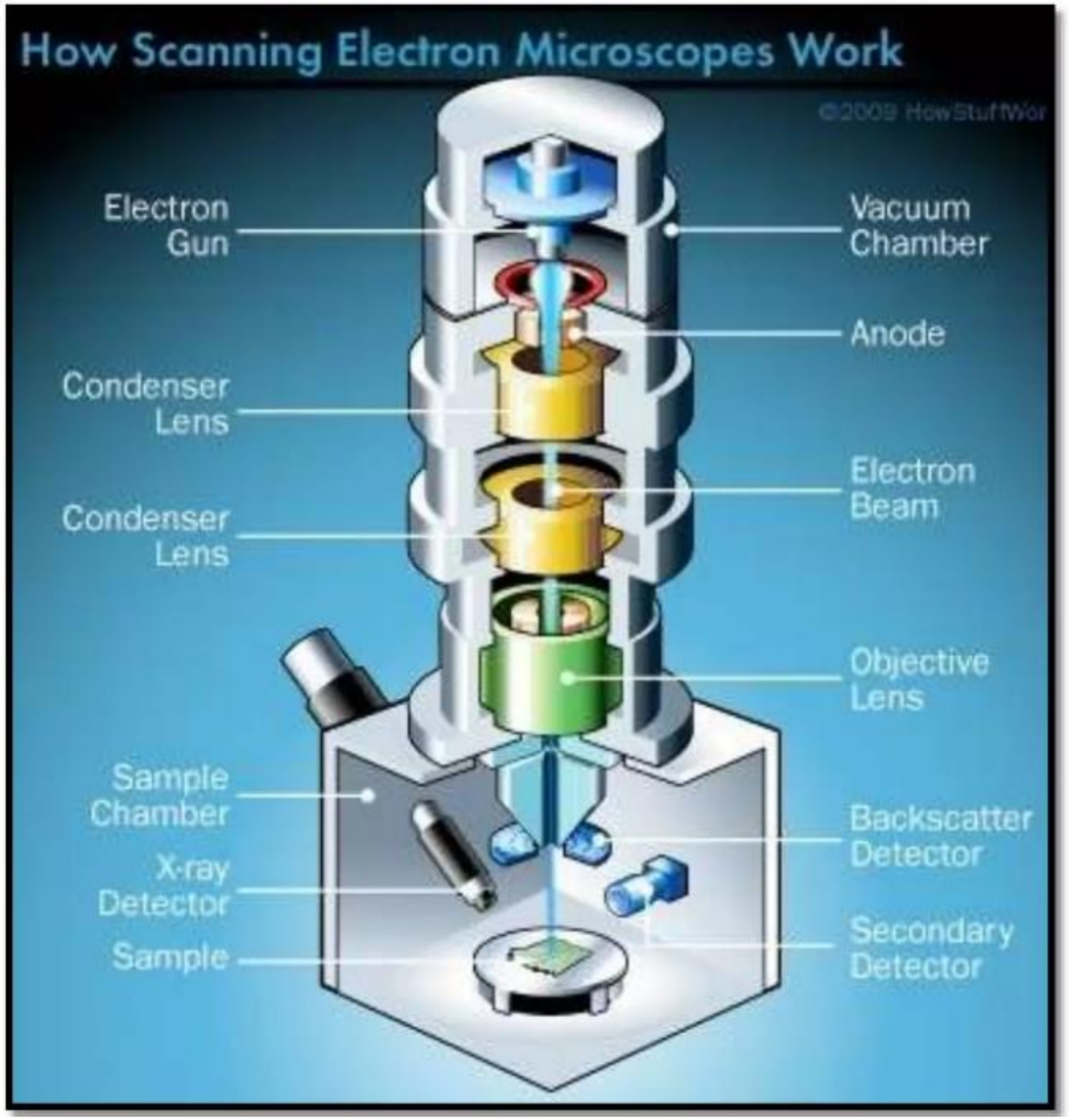


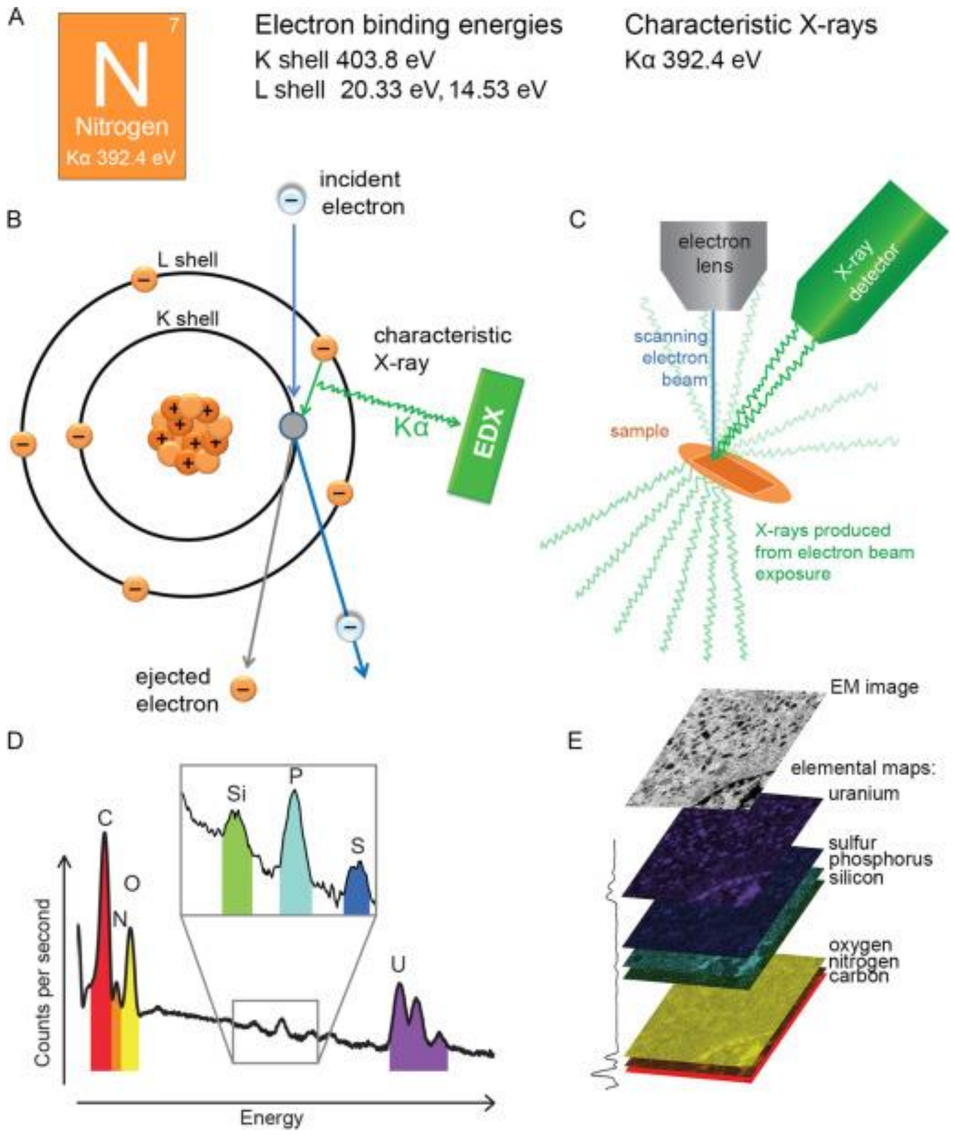


Granuloma: aggregate of macrophages in response to inflammation caused by unremoved foreign body. Activated macrophages can merge to form an “epithelioid” barrier[1].

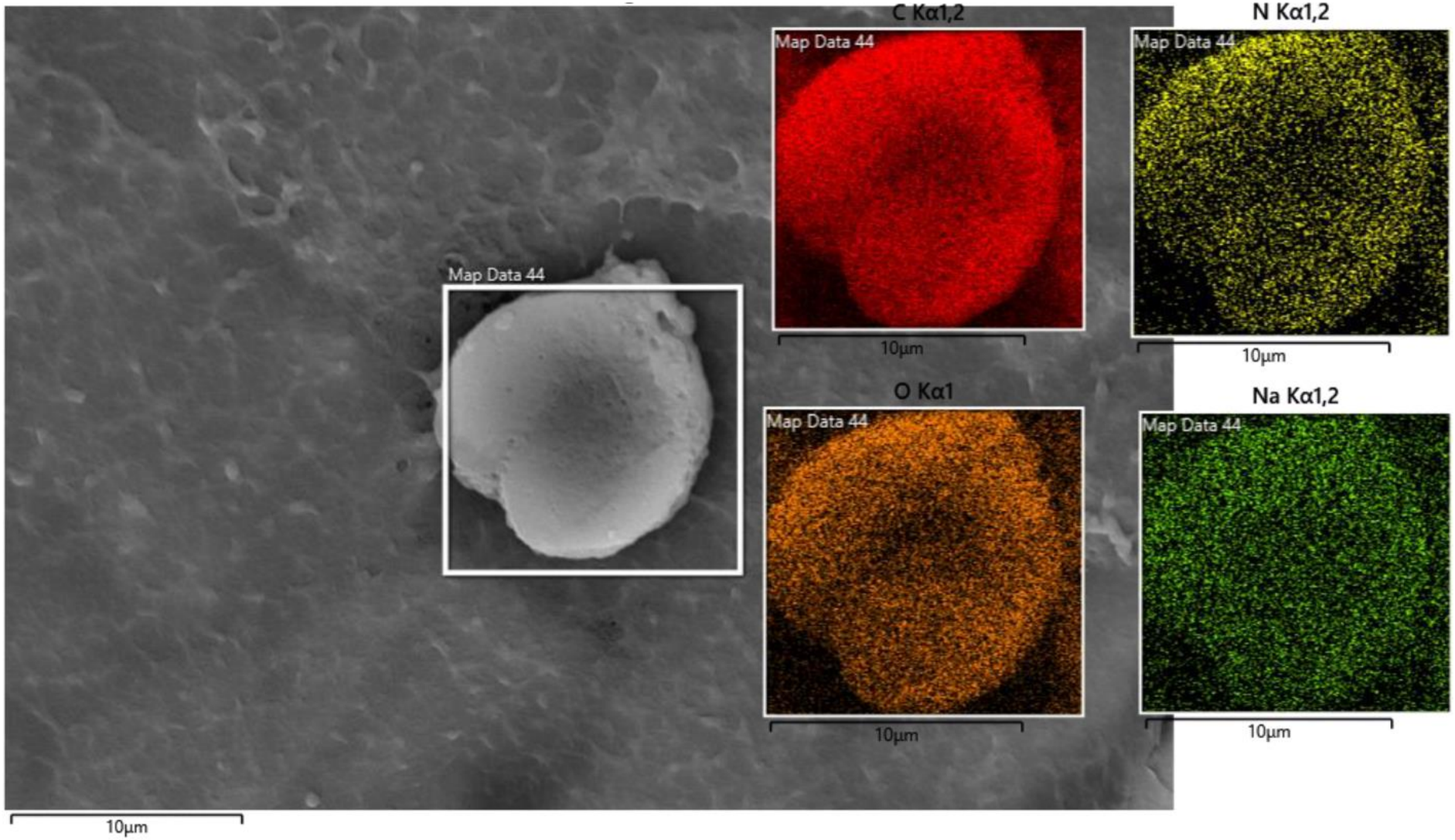
1. https://www.ncbi.nlm.nih.gov/books/NBK554586/

SEM-EDX

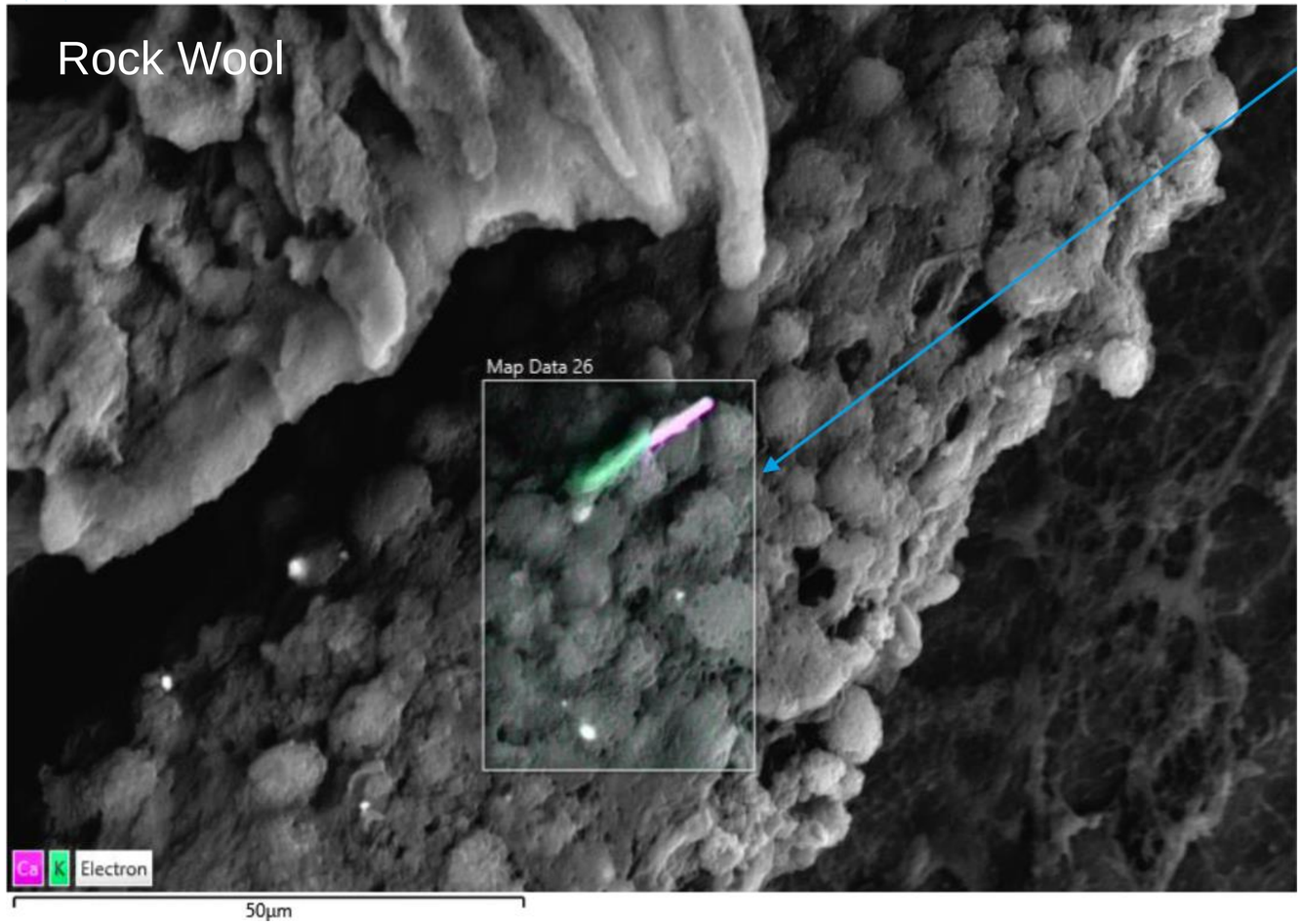

How Scanning Electron Microscopes Work
©2009 HowStuffWor
Electron Gun
Vacuum Chamber
Anode
Condenser Lens
Electron Beam
Condenser Lens
Objective Lens
Sample Chamber
Backscatter Detector
X-ray Detector
Secondary Detector
Sample

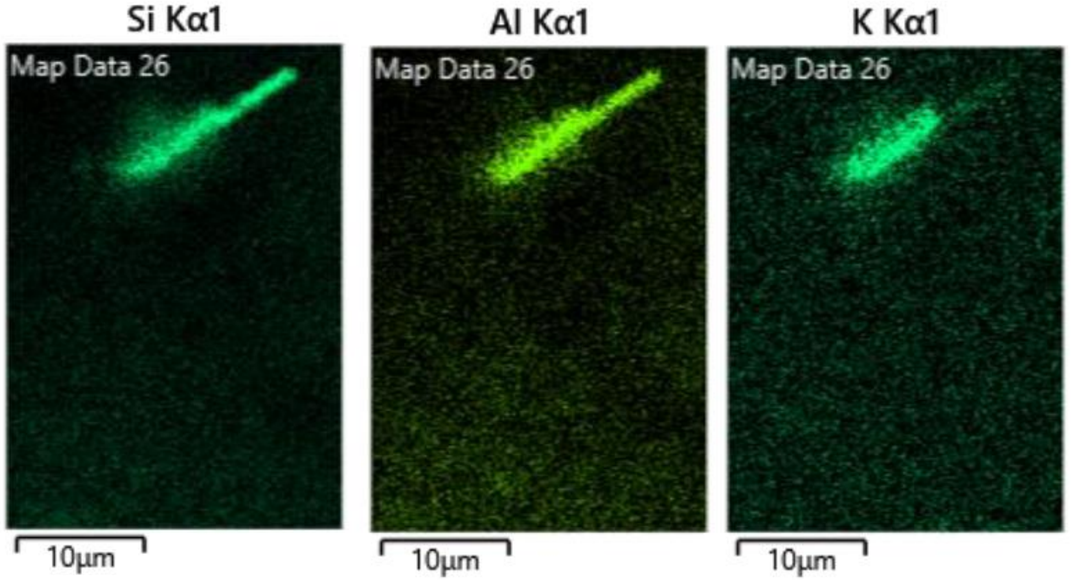

A
7
N
Nitrogen
Kα 392.4 eV
Electron binding energies
K shell 403.8 eV
L shell 20.33 eV, 14.53 eV
Characteristic X-rays
Kα 392.4 eV
B
incident electron
L shell
K shell
characteristic X-ray
Kα
EDX
ejected electron
C
electron lens
X-ray detector
scanning electron beam
sample
X-rays produced from electron beam exposure
D
Si
P
S
C
O
N
U
Counts per second
Energy
E
EM image
elemental maps:
uranium
sulfur
phosphorus
silicon
oxygen
nitrogen
carbon

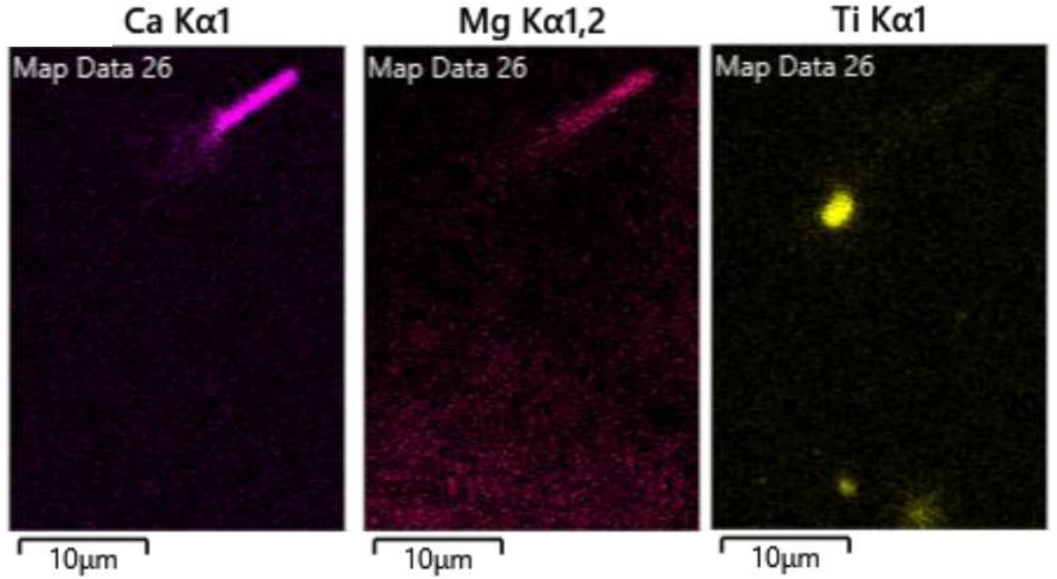
SEM-EDS analysis of Macrophages without MMVF



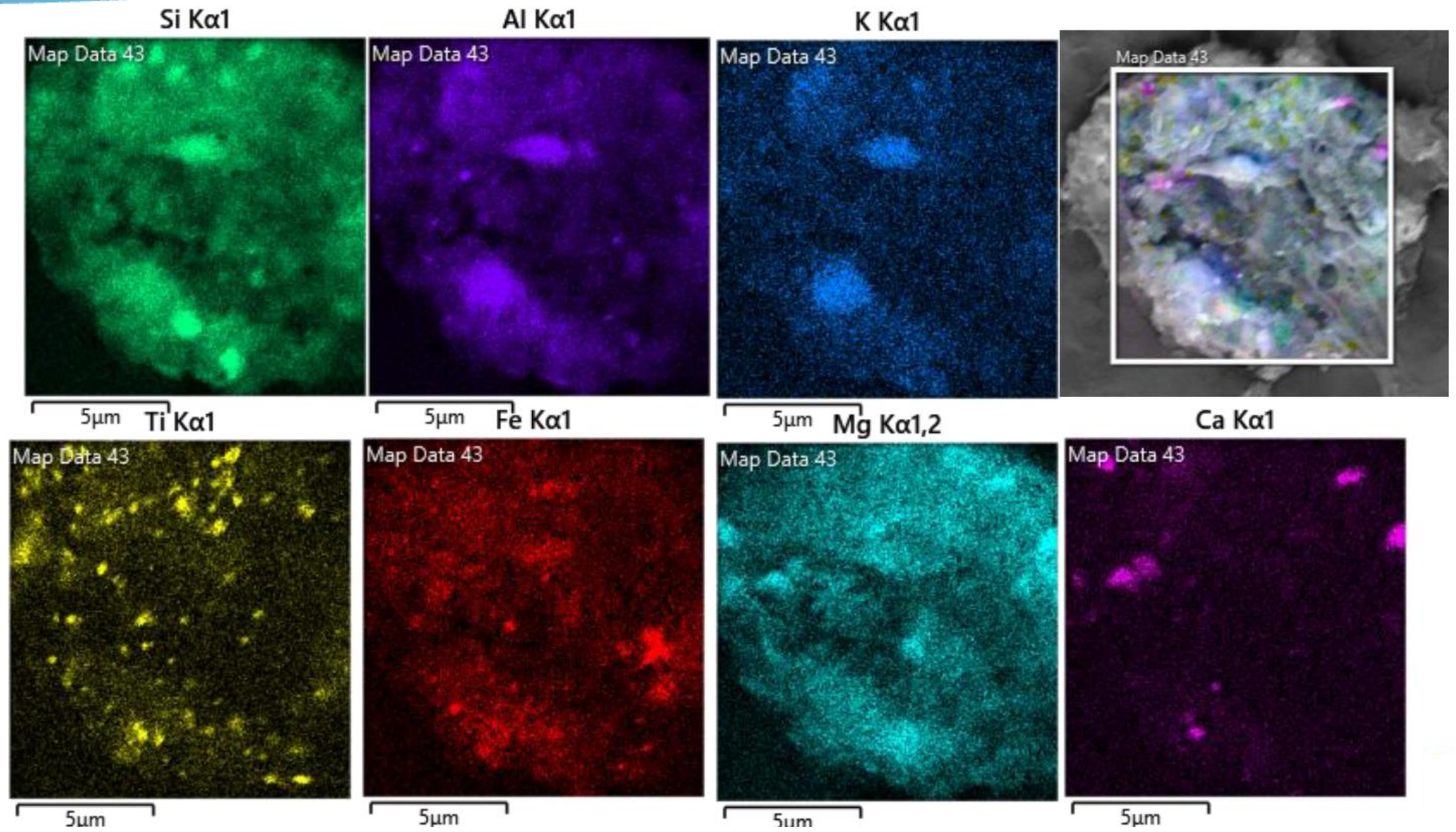
Rock wool in AlveolAir™ - Macrophages







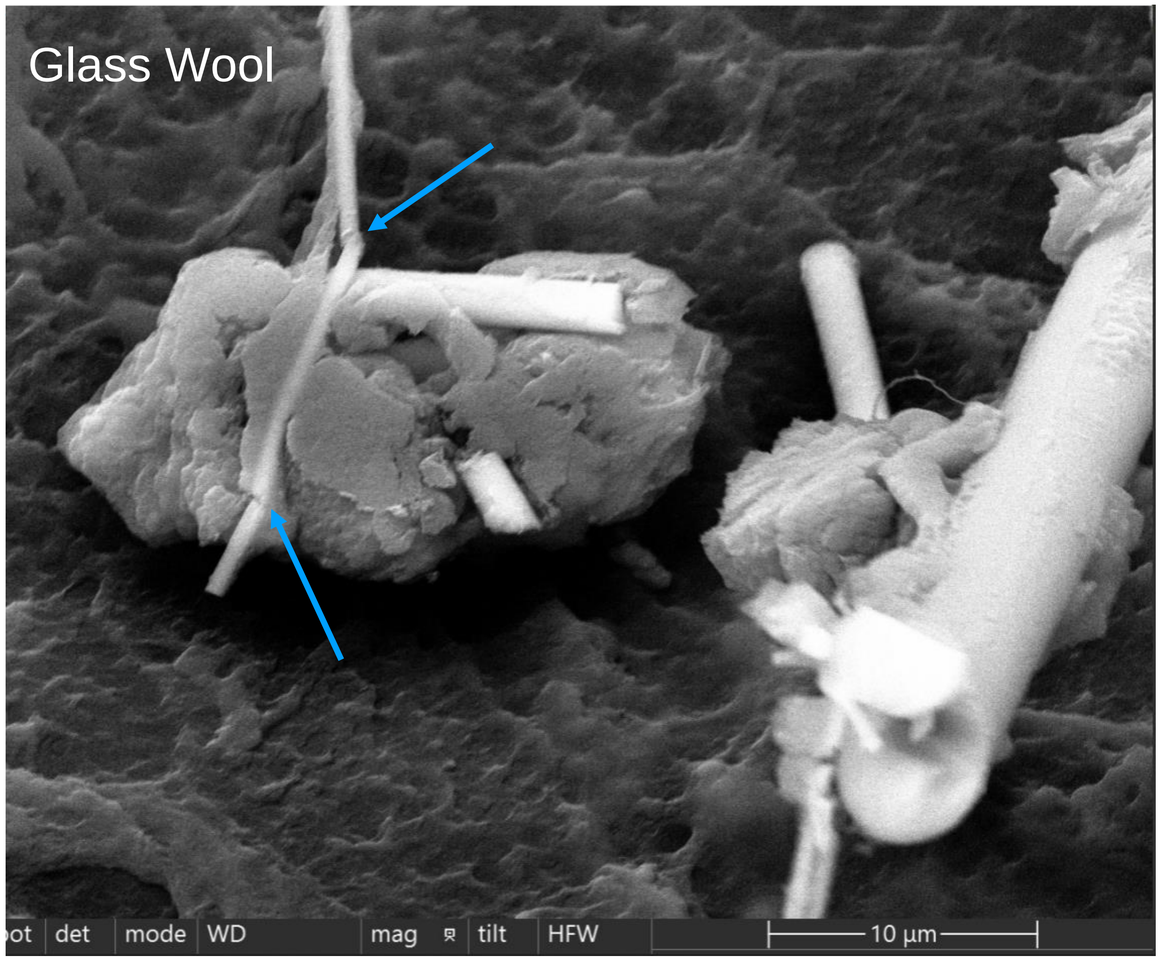
Rockwool is dissolved by alveolar macrophages

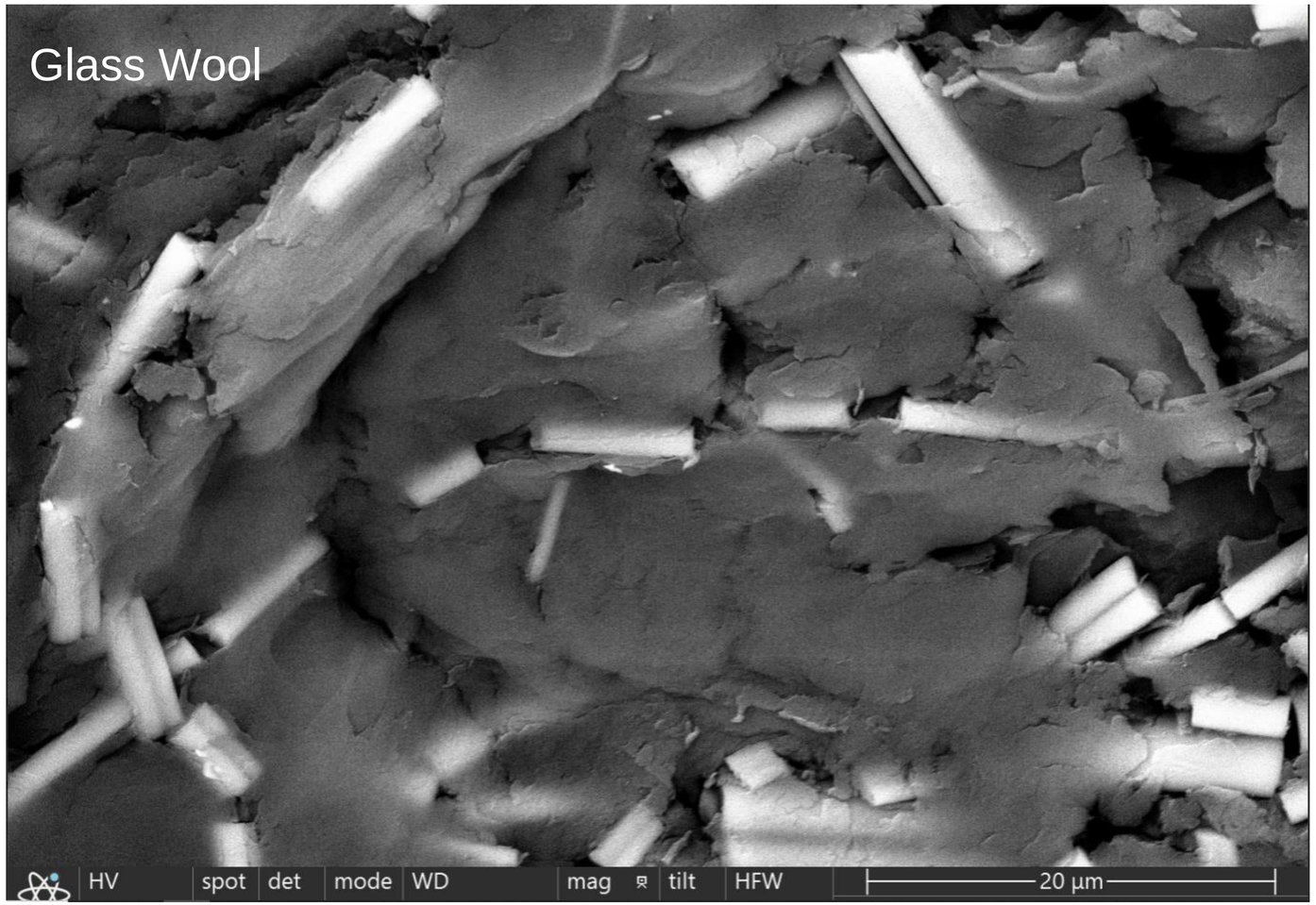


- Alveolar macrophages dissolve rockwool fibers, consistent with *in vivo* results

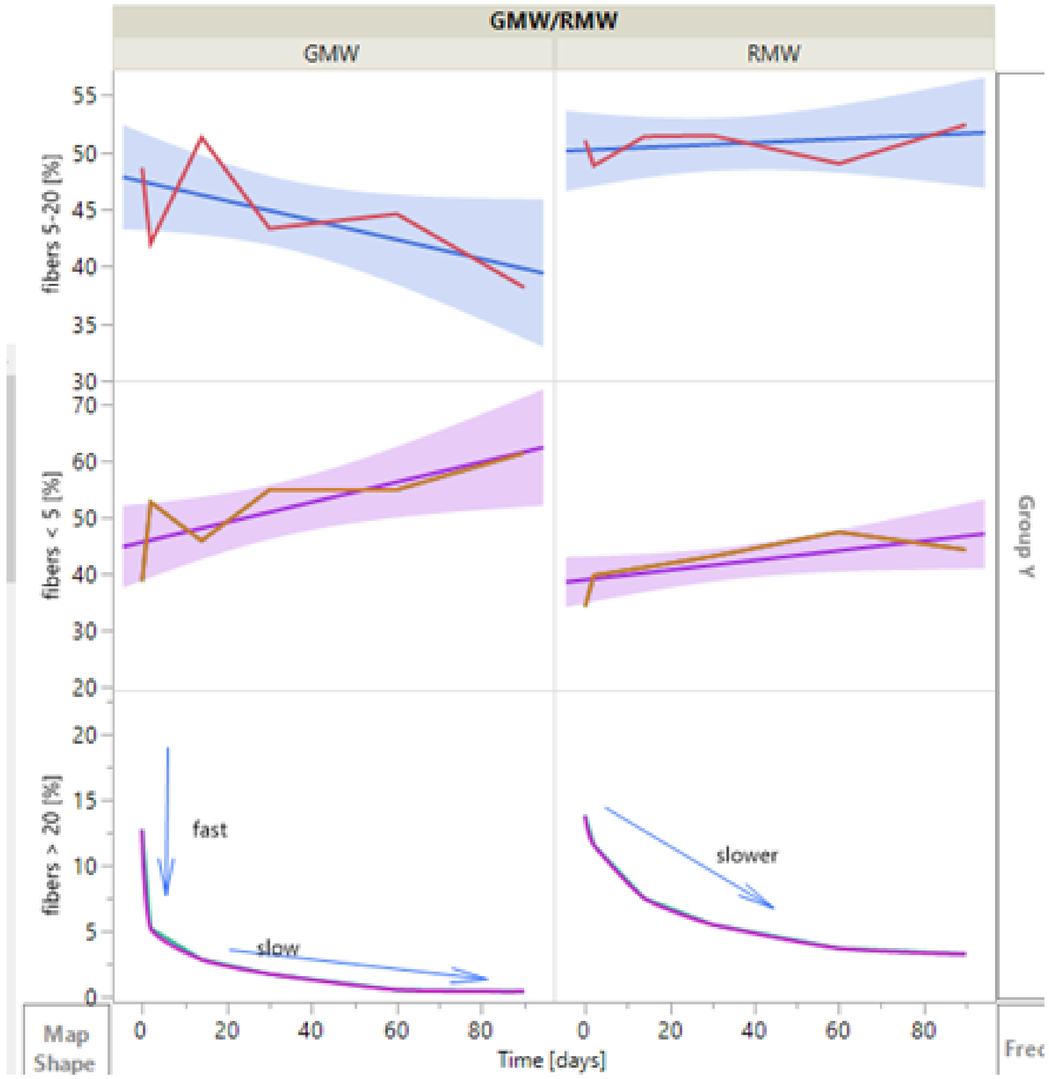
Hesterberg et al., Fundamental and applied toxicology, 1995

Glass wool is broken by alveolar macrophages



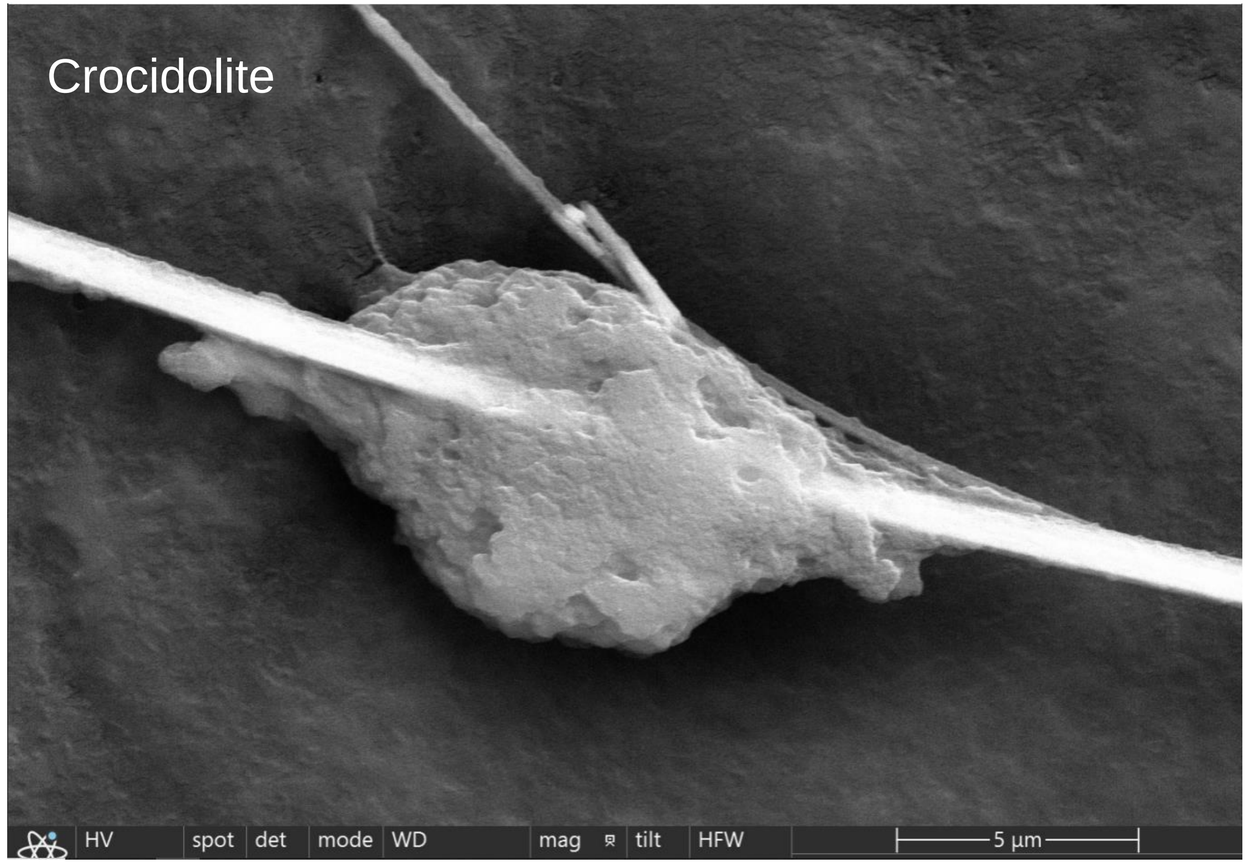


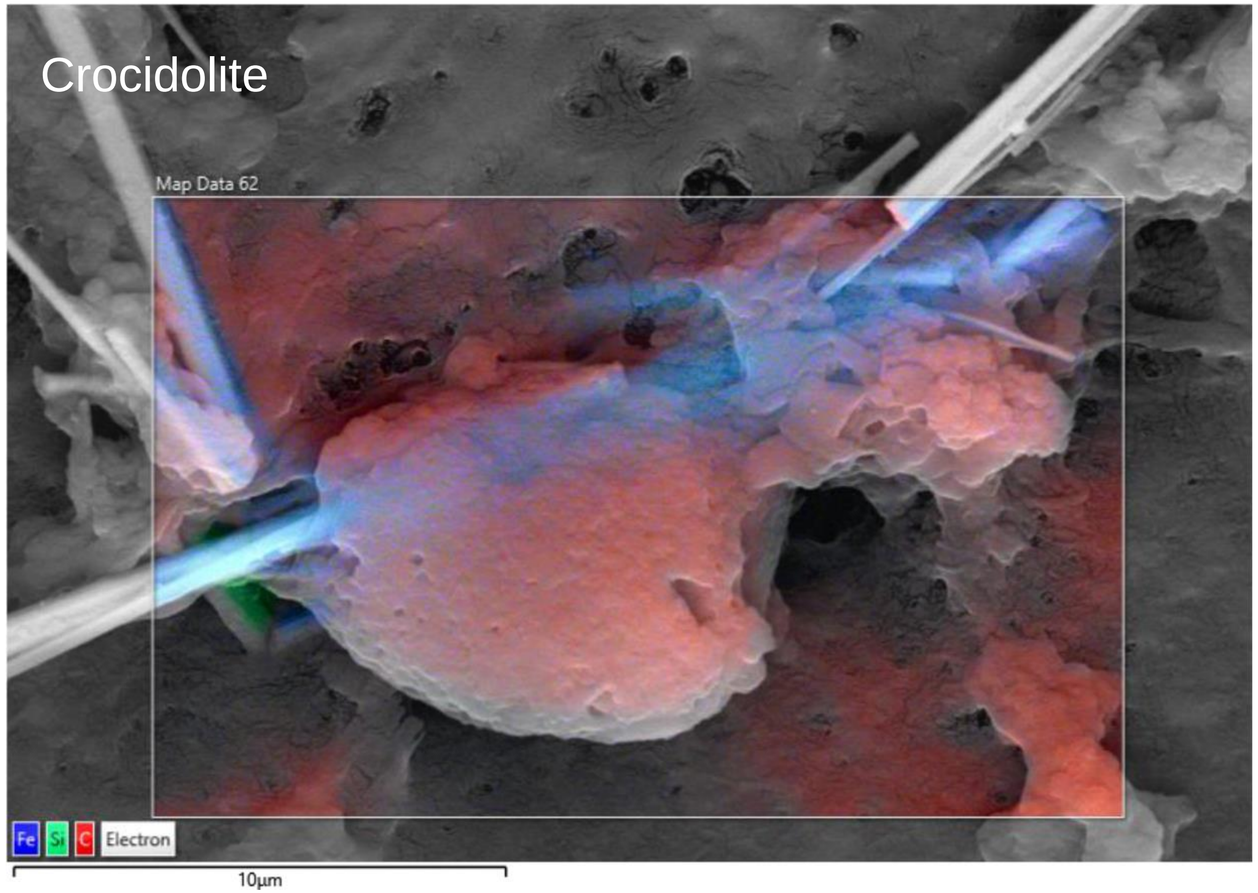
Glass wool is broken by alveolar macrophages



- Alveolar macrophages break down glass wool, consistent with *in vivo* data.

Crocidolite – Macrophages interactions





Conclusion

- Nasal, Bronchial, Small-Airways and Alveolar 3D human Models are useful tools to evaluate effect of inhaled xenobiotics on:
  - ✓Local tolerance
  - ✓Respiratory absorption
  - ✓Mucociliary clearance
  - ✓Mucosal inflammation
  - ✓Respiratory disease induction (lung fibrosis, metaplasia)

- Alveolar macrophages replicate in vivo results of MMVF dissolution, breakage and phagocytosis when co-cultured with AlveolAir™

- Assays need to be developed on emergent immunocompetent models integrating alveolar macrophages, dendritic cells and neutrophils.

EPITHELIX
IN VITRO RESPIRATORY SOLUTIONS

Thank you for your attention

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