Precision-cut Lung Slices (PCLS): Long-term evaluation of Nanoparticles

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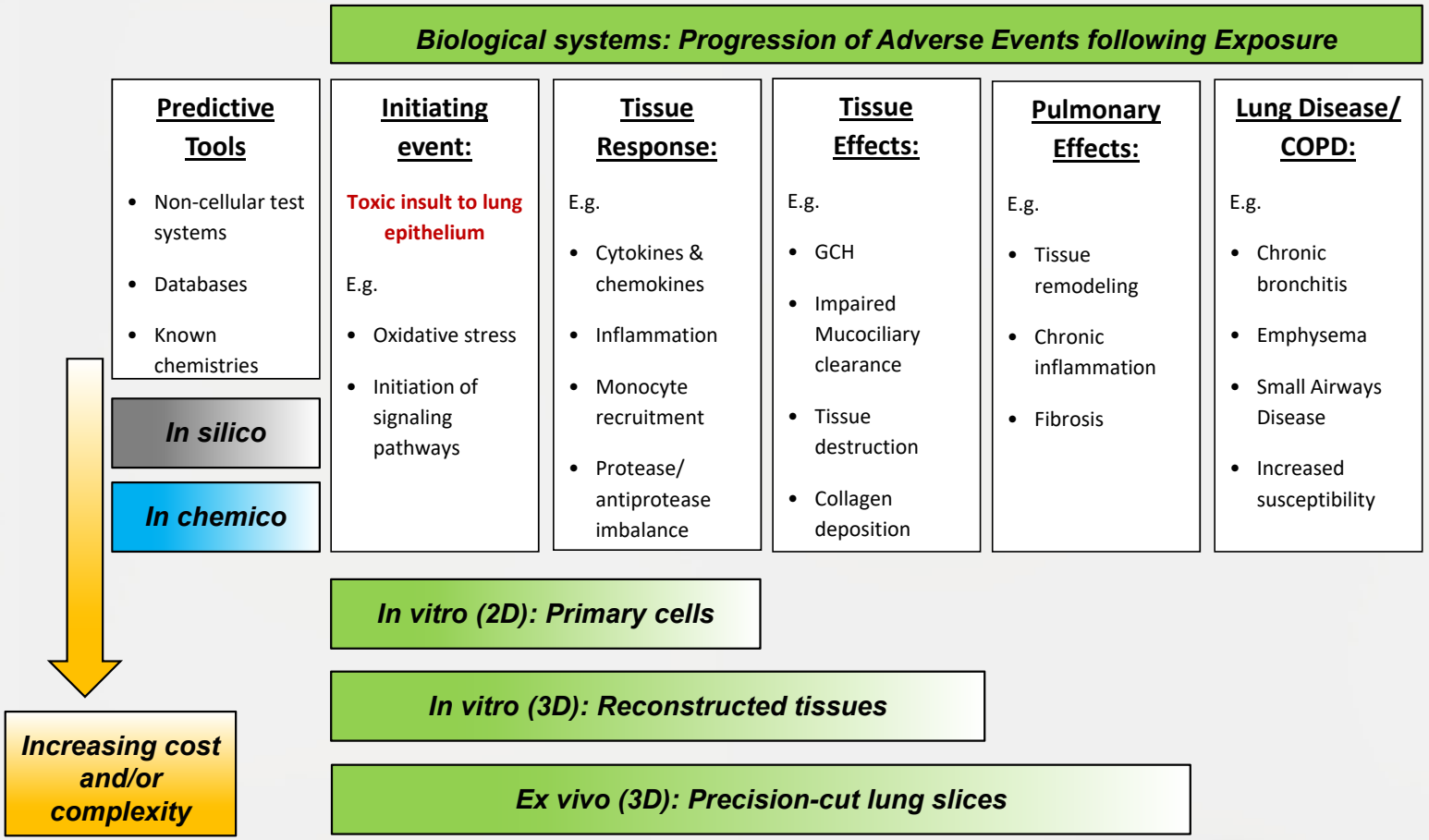
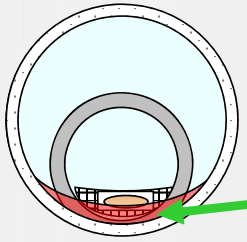
Adverse Respiratory Events & Choice of System



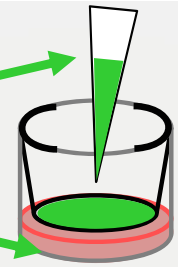
Exposure Methods: Solubles & Aerosols

Solubilized material, applied to PCLS

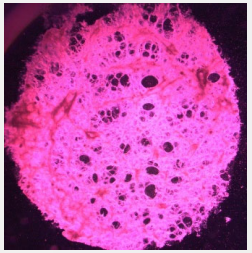
Roller culture



ALI/submersion culture



Tissue insert at air liquid interface (ALI)

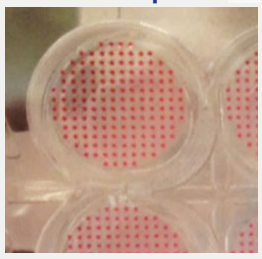


Novel: Digital Dispensing

TECAN/HP D300

ALI/submersion culture

3 nL droplets



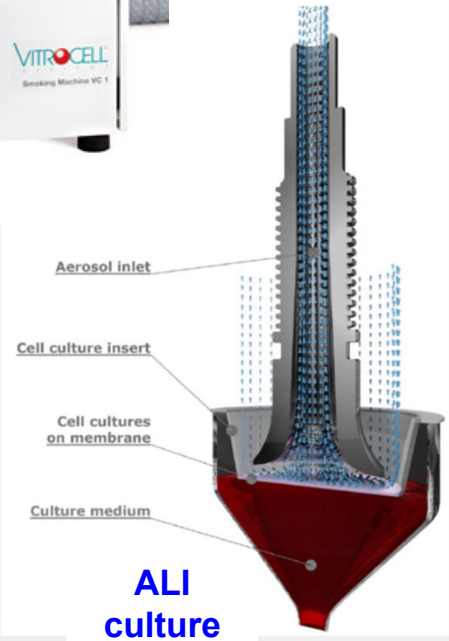
Accurate delivery to ALI

- Hybrid technology?
- DMSO or aqueous-based solutions
- Minimal volume delivery

Smoke/Aerosol /Gas Delivery

VITROCELL® VC-1

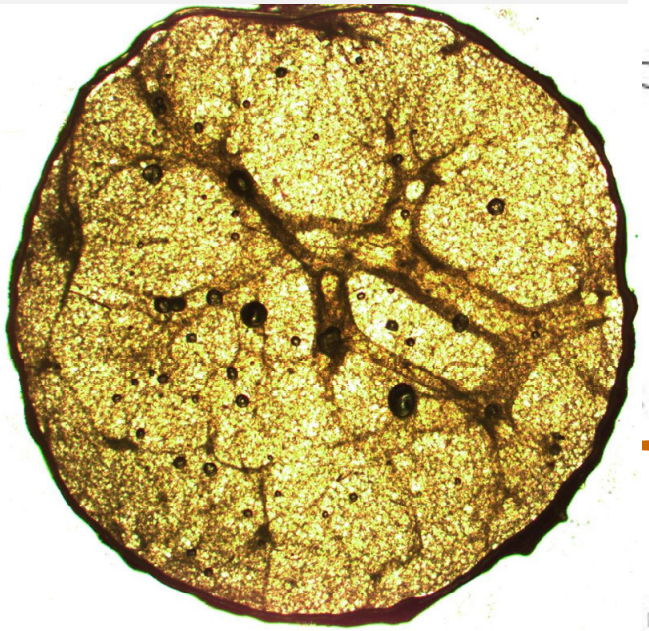
- Most relevant!
- Dosimetry?
- Cost!

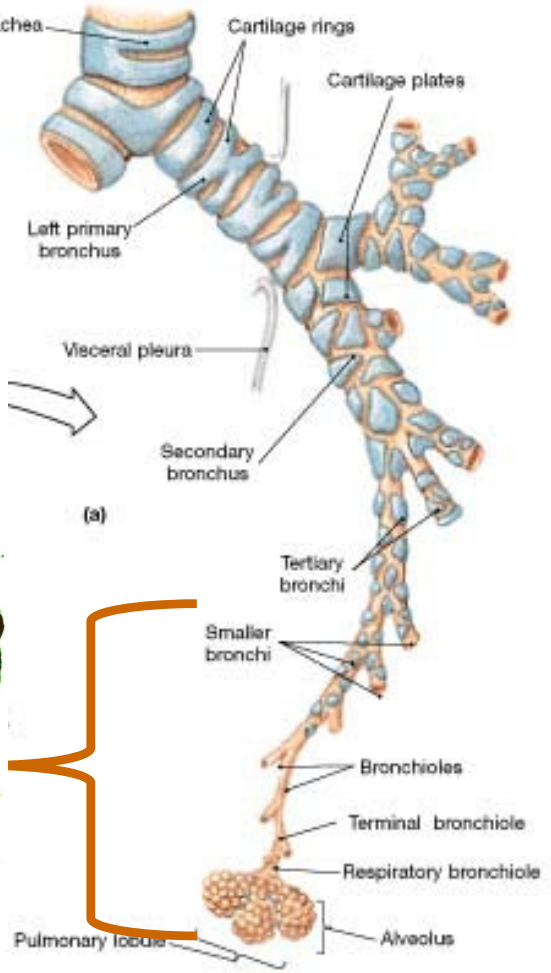


ALI culture

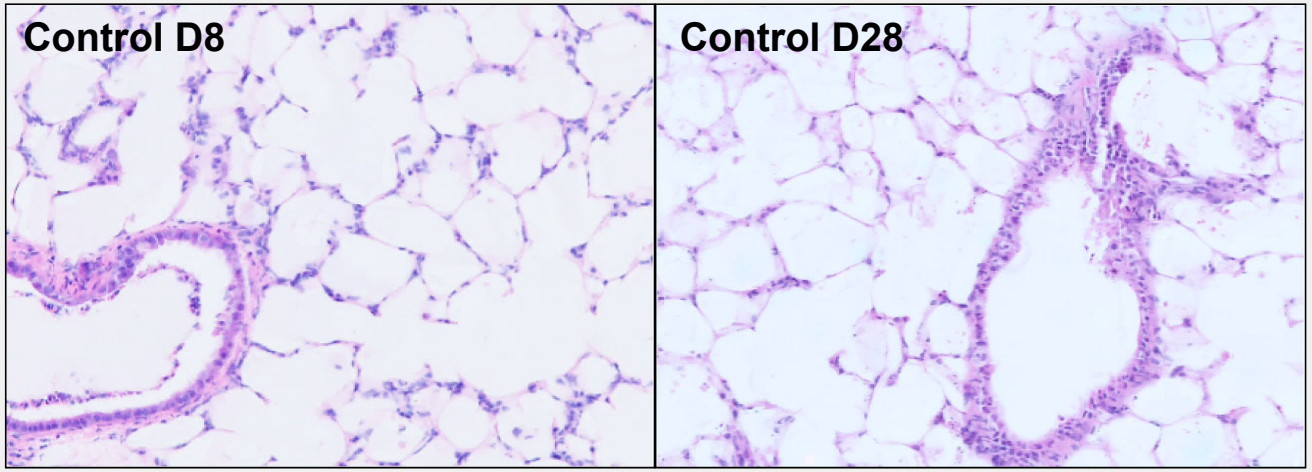
In Vitro/Ex vivo Models: PCLS

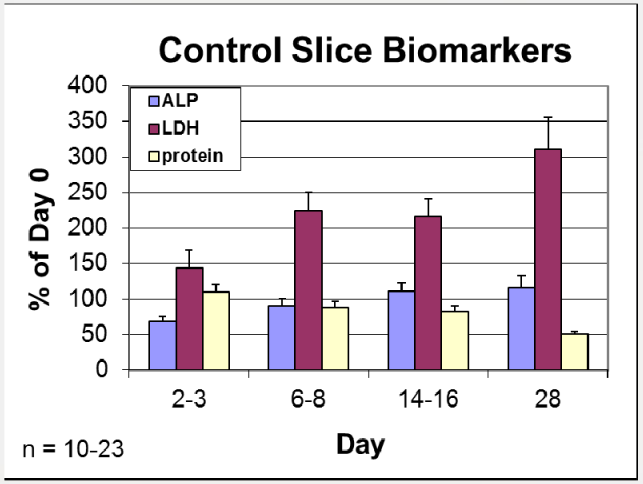
PCLS can represent all lung regions present in tissue source

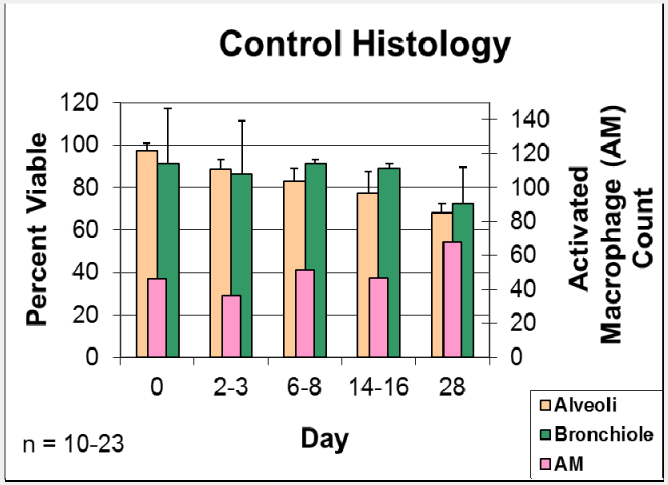




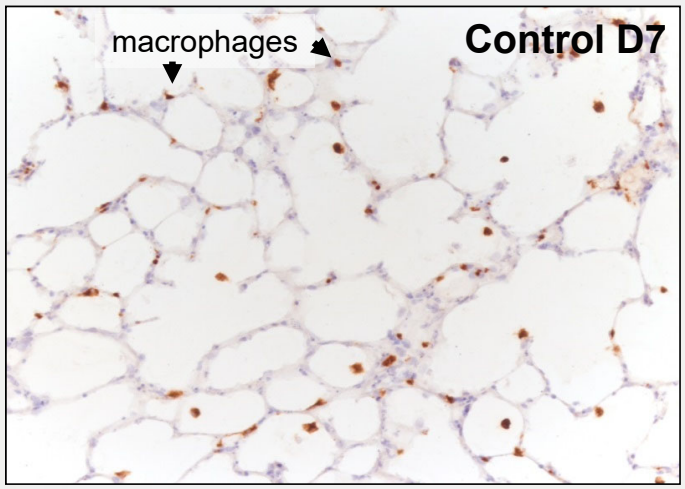
PCLS: Long Term Culture (e.g. rat PCLS)

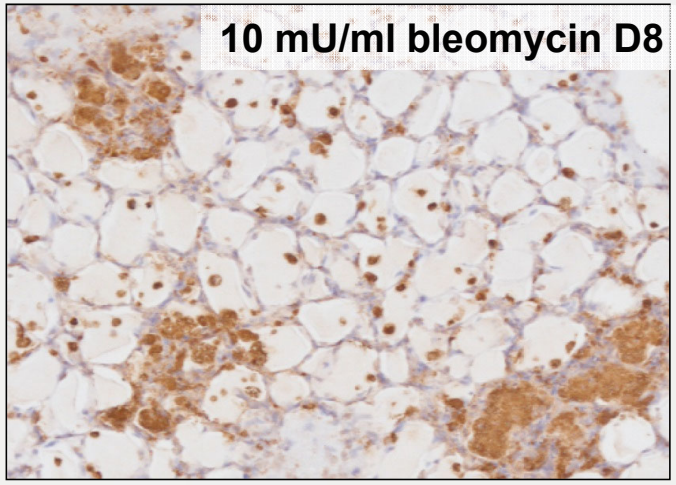


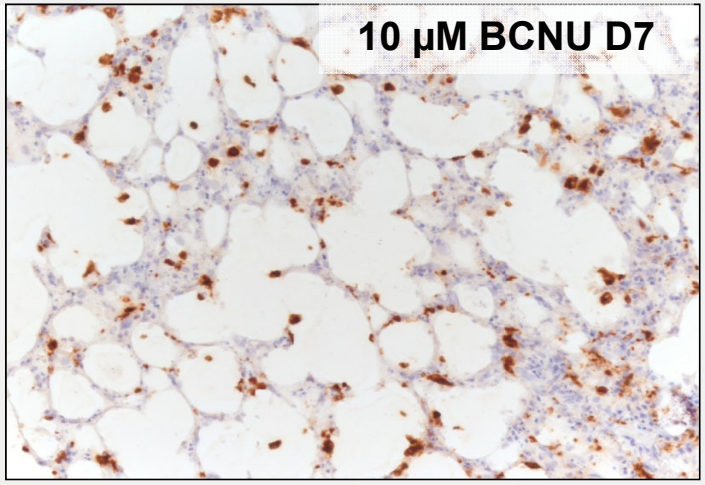




PCLS: Compound-induced Macrophage Activation







Negative control PCLS exhibit a **baseline ED-1 staining of macrophages**. Macrophages are maintained in cultures for 28 or more days

Bleomycin treatment results in **patches of activated macrophages filling alveolar spaces**; many solitary macrophages also seen

BCNU (carmustine) exposure shows **numerous macrophages**, many of which have **infiltrated alveolar walls** mimicking interstitial pneumonitis

Rat PCLS: ED-1 IHC Stain

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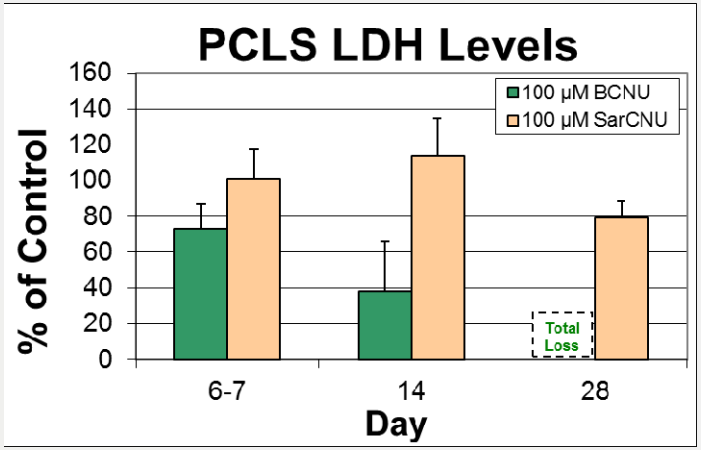
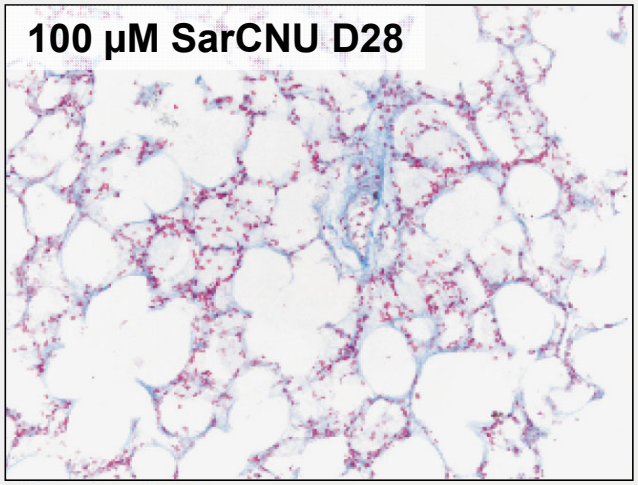
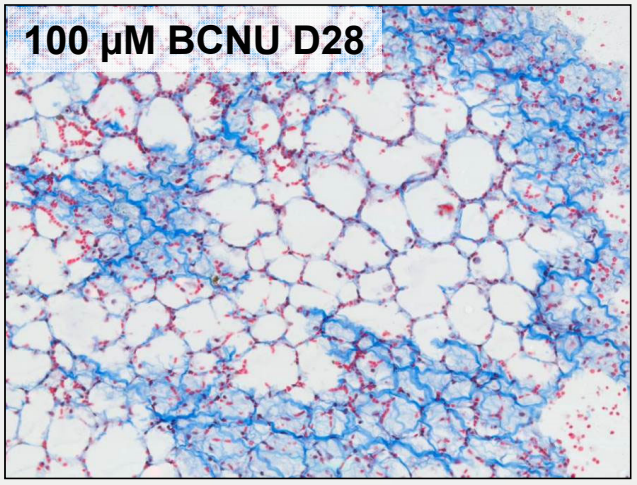
PCLS (rat): BCNU vs SarCNU Comparison

- **BCNU** has been reported to cause **lung damage and fibrosis** in 20-30% of the patients receiving it (Weiss, Poster et al. 1981)…
- More recently an analog of BCNU, 2-Chloroethyl-3-sarcosinamide-1-nitrosurea (**SarCNU**), has been shown to be well-tolerated, more effective, and **less toxic**…







Activated Macrophages in Lung Slices				
		Counts [a]		
Compound	µM	Day 6-7	Day 14	Day 28
Vehicle	0	52	48	59
BCNU	1	60	94**	-
	10	67*	71*	-
	100	66	102**	81*
SarCNU	1	56	52	-
	10	61	57	-
	100	69*	76*	71

[a] Means of 3 measurements on 3-4 replicates
* $p<0.05$ ** $p<0.01$

Quartz Silica and Silicosis

https://www.greenscreenchemicals.org/gs-assessments/chemical/14808-60-7

MIN-U-SIL® 5
GROUND SILICA
CAS# 14808-60-7

CHARACTERISTICS:

MIN-U-SIL® 5 is a natural, fine ground silica with high purity. The consistent pH and narrow size distribution of MIN-U-SIL® 5 allows very high loading with minimal effect on viscosity and cure rate. MIN-U-SIL® 5 is inert, and offers increased shore durometer, reduced shrinkage, improved thermal conductivity, increased density, improved oil resistance, and high dielectric strength.

Specifications:

Particle Size	
***5 microns or less**	**96%**
Median Size	**1.6 microns**
***Retained on 325 mesh**	**.004%**

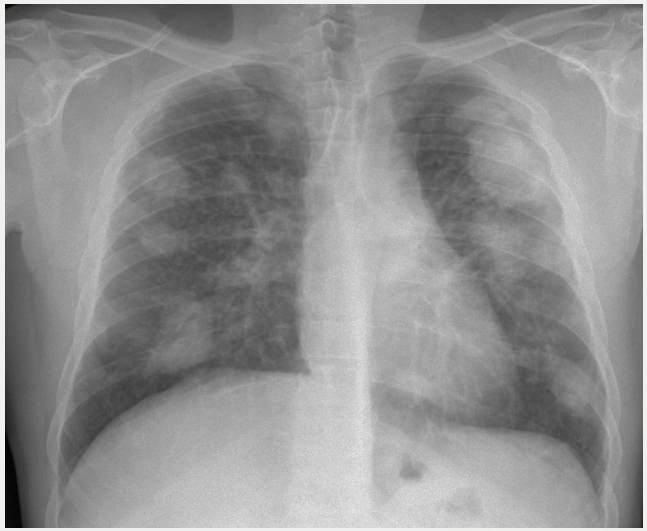
http://www.wrchem.com/wp-content/uploads/2015/11/MIN-U-Sil-5-Ground-Silica-TDS.pdf

- CAS#: 14808-60-7
- Chemical Name: **Quartz**
- Primary Applications: Hydraulic Fracturing, Processing Aids and Additives
- Quartz is a chemical that has many uses, including in glass manufacturing, ceramics, foundry, abrasives, hydraulic fracturing, furnaces, fillers, paints, and filtration.

Effects of Silicosis

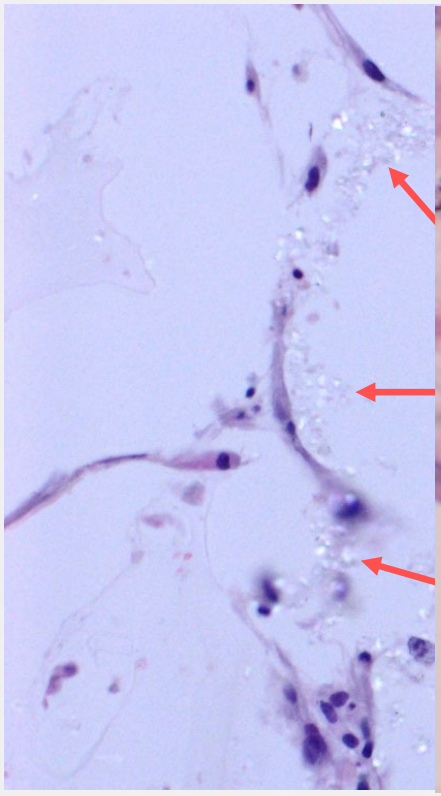
- **Lung cancer** – Silica has been classified as a human lung carcinogen.
- **Bronchitis/Chronic Obstructive Pulmonary Disorder.**
- **Tuberculosis** – Silicosis makes an individual more susceptible to TB.
- Scleroderma – a disease affecting skin, blood vessels, joints and skeletal muscles.
- Possible renal disease.
- Respiratory failure, which may eventually lead to death.

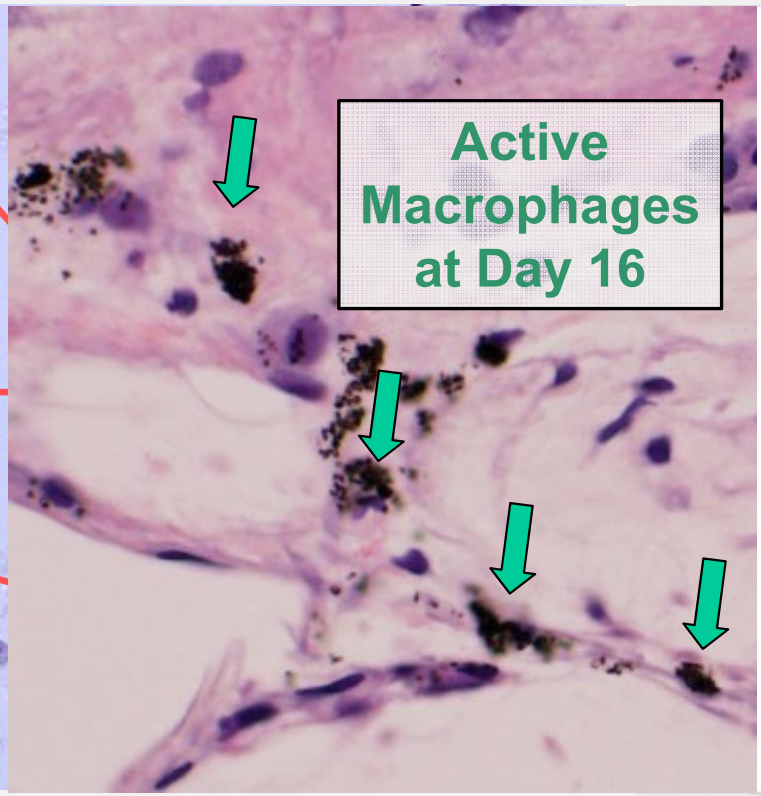
https://www.osha.gov/Publications/silicosis.html

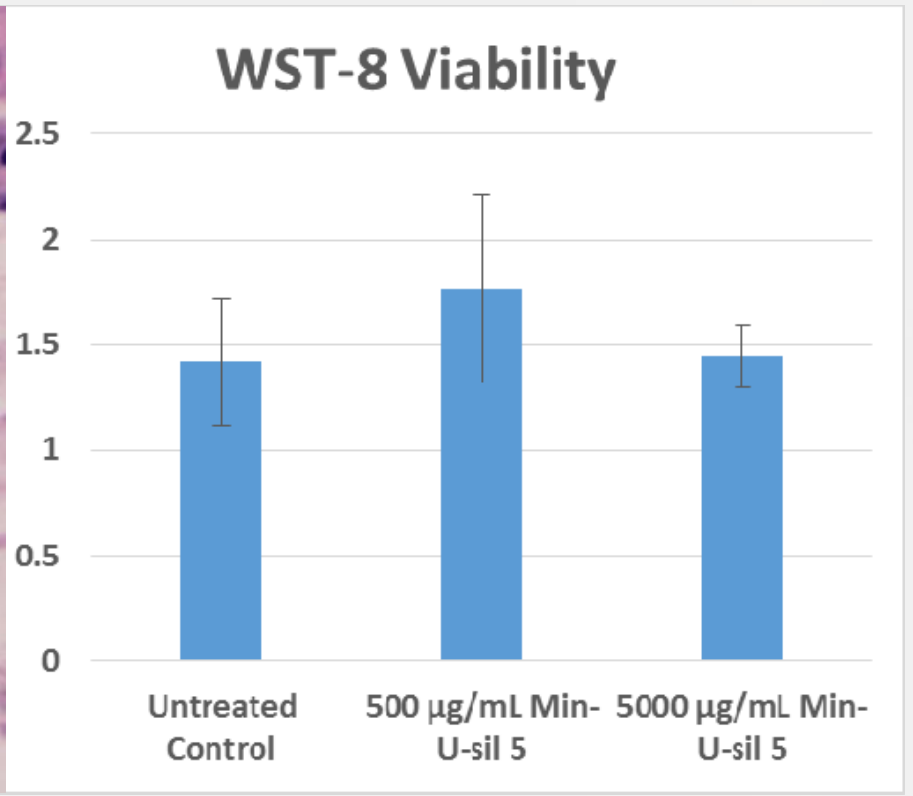


Pilot Study: Min-U-sil 5 Exposure to HuPCLS

500 µg/mL Min-U-sil 5, 16 days (daily refeedings)





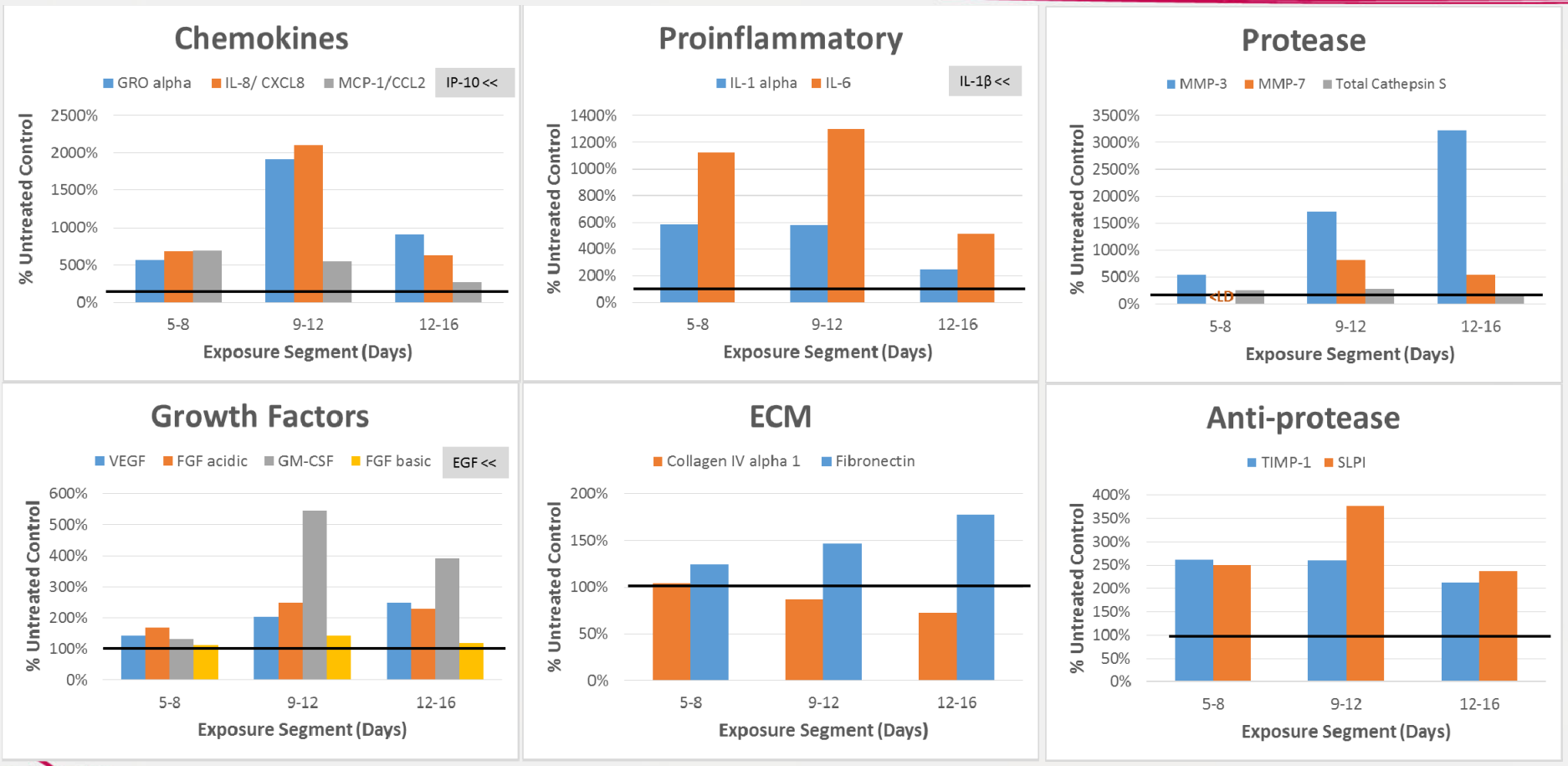


Pathologist:

- Particles seemingly inert
- No macrophages engulfing
- No overt toxic events seen

No overt viability loss

500 µg/mL Min-U-sil 5 Exposure to HuPCLS



Note: values above the highest standard were extrapolated by Luminex xPonent software

Summary

- **PCLS can be maintained for weeks, allowing chronic exposure and evaluation of long-term effects**
  - Native architecture for histological observations
- **Demonstrated utility:**
  - Differences in species sensitivity (not shown) and analog potency
  - Concentration-dependent reversibility of inflammation (not shown)
- **Response to silica exposure**
- Prolonged PCLS exposure to MinUsil 5 resulted in prolonged elevation of inflammatory markers, but no loss of overall viability yet offering the processes such as chronic inflammation

- Nanoparticle risk scenarios include repeat and/or prolonged exposures that may result in chronic inflammatory states – potentially leading to pulmonary fibrosis.
- The PCLS test system offers a long-term culture period during which multiple, immune-competent cell types, and chronic inflammatory processes can be monitored to study the effects of nanoparticles.

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Acknowledgements

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- Simon Fogarty (Tecan) Christi Dudenhofer and David Ochse (HP)

Questions?

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