



IMPACTS OF INHALED ENVIRONMENTAL PARTICLES ON MACROPHAGE INFLAMMATORY FUNCTIONS

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PURPOSE OF THIS PROJECT

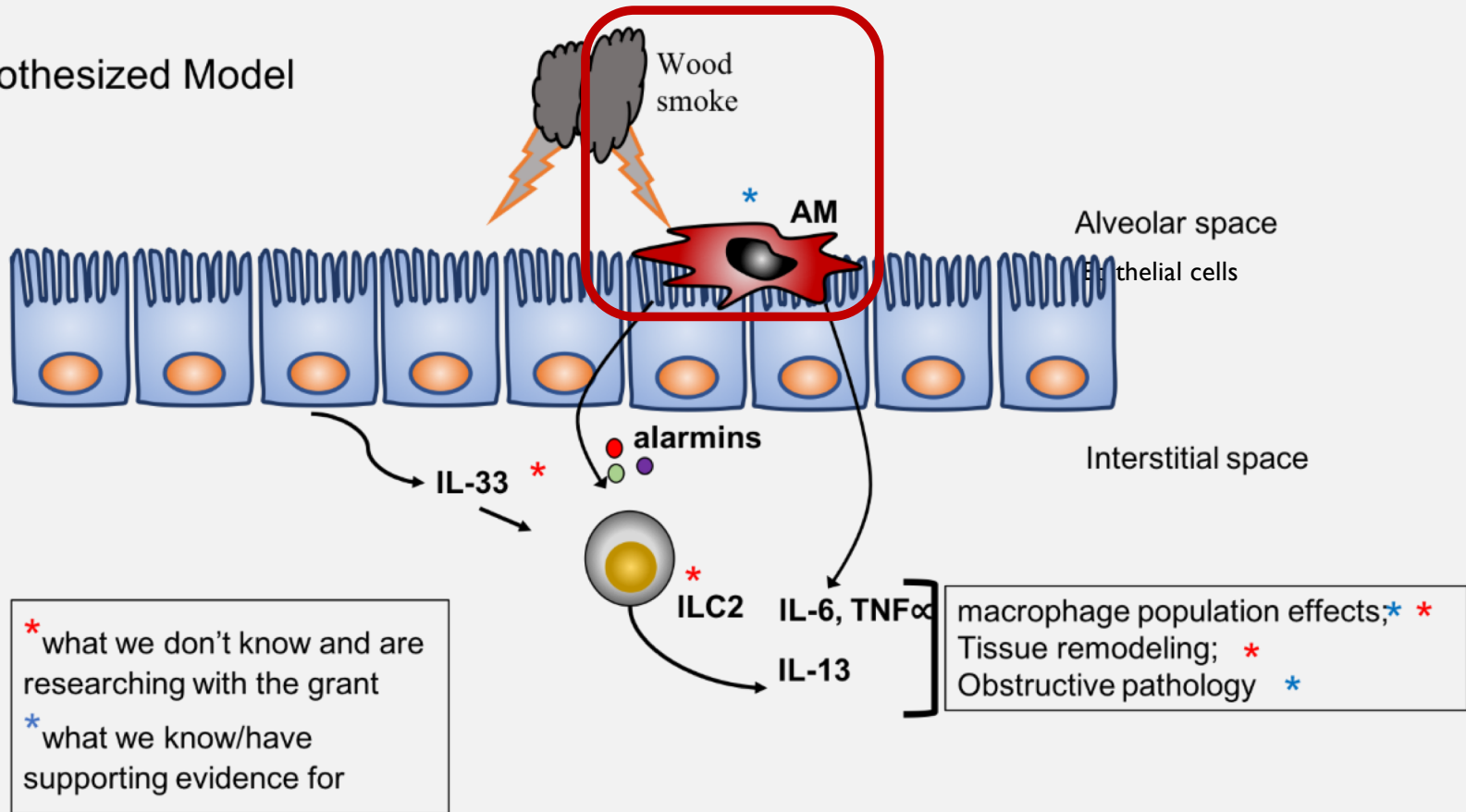
- Assess the effects of inhaled environmental particles on macrophage function and in turn how it affects immunity.
- Particle exposed macrophages ability to perform efferocytosis
 - Efferocytosis: the process by which apoptotic cells are recognized and cleared by phagocytic cells

HYPOTHESIS

- Alveolar macrophages represent not only a mechanistic link between wildfire smoke exposure and adverse respiratory health effects, but also a means to manipulate the IL-33/ILC2 signaling axis for therapeutic benefit.
- **Wood smoke exposures alter macrophage functions resulting in an increase in inflammation that will affect the local environment and result in diminished lung functions.**



Hypothesized Model



WOOD SMOKE EXPOSURE

- Inhalation causes adverse effects on human health
- Developing a mouse model is important to understand human pathology
- This exposure is modeled after the 2017 study on wildfire smoke exposure in Seeley Lake (Orr et al. 2020)

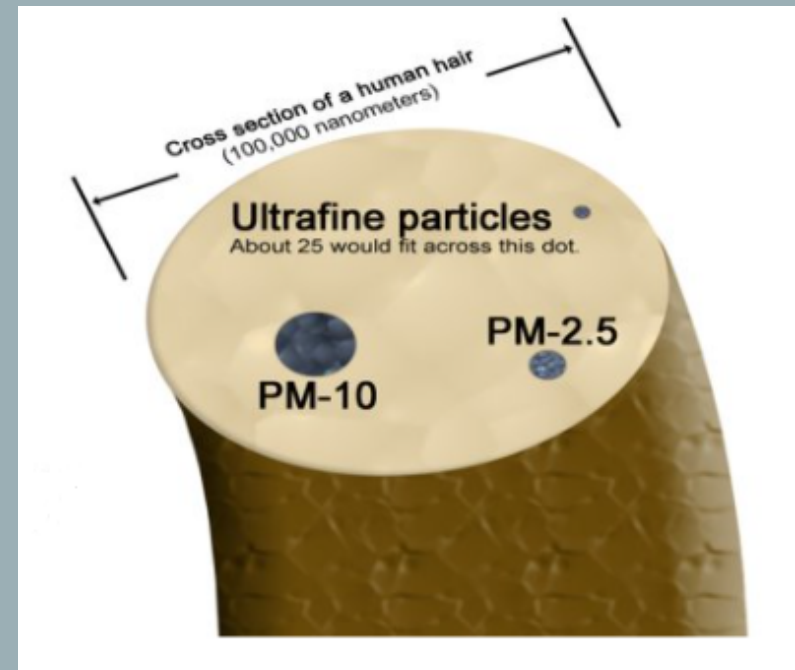


CRYSTALLINE SILICA

- We are using silica as a comparison to wood smoke
- Natural material found in dusty trades, and in many popular products
- When inhaled, silica can penetrate deep into the lungs can cause silicosis, lung cancer, kidney disease, and chronic pulmonary obstructive disease (United States Department of Labor N/A)

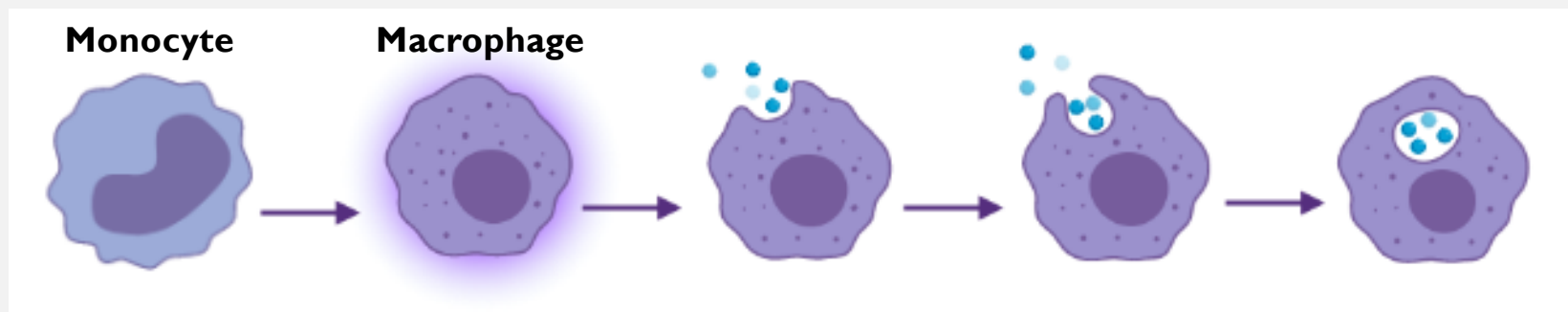
PARTICULATE EXPOSURES

- Particles less than $2.5\mu m$ in diameter pose the greatest risk to human health (EPA 2021)
- Wood smoke particles = $2.5\mu m$
- Crystalline silica particles = $0.8\mu m$



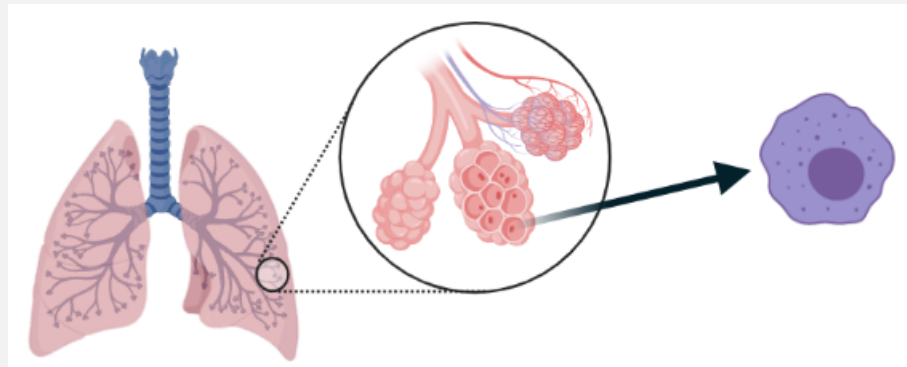
MACROPHAGES

- Type of white blood cell in the immune system that specialize in phagocytosis of harmful organisms and particles
- Derived from monocytes



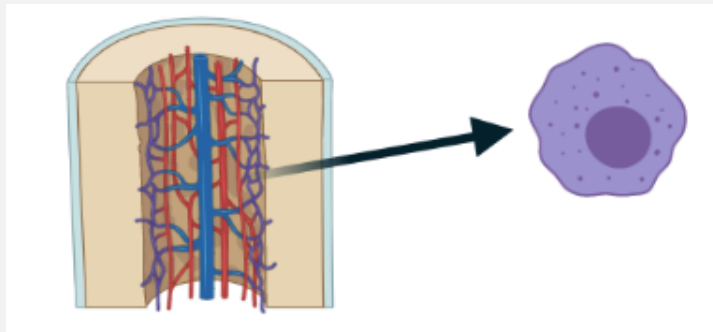
ALVEOLAR MACROPHAGES

- A key component when looking at respiratory immunity
- Play an important role in both the induction and resolution of inflammatory responses



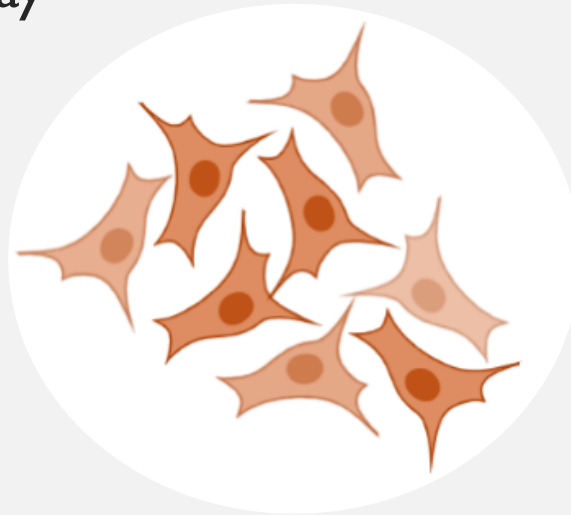
BONE MARROW DERIVED MACROPHAGES

- Macrophage cells that are derived from bone marrow cells *in vitro*
- More abundant in mice models



C10 CELLS

- Alveolar epithelial cells
- Cultured C10 cells to use as apoptotic cells for the efferocytosis assay



Efferocytosis Assay

CSFE and CytoTell Blue stains
will label the cells

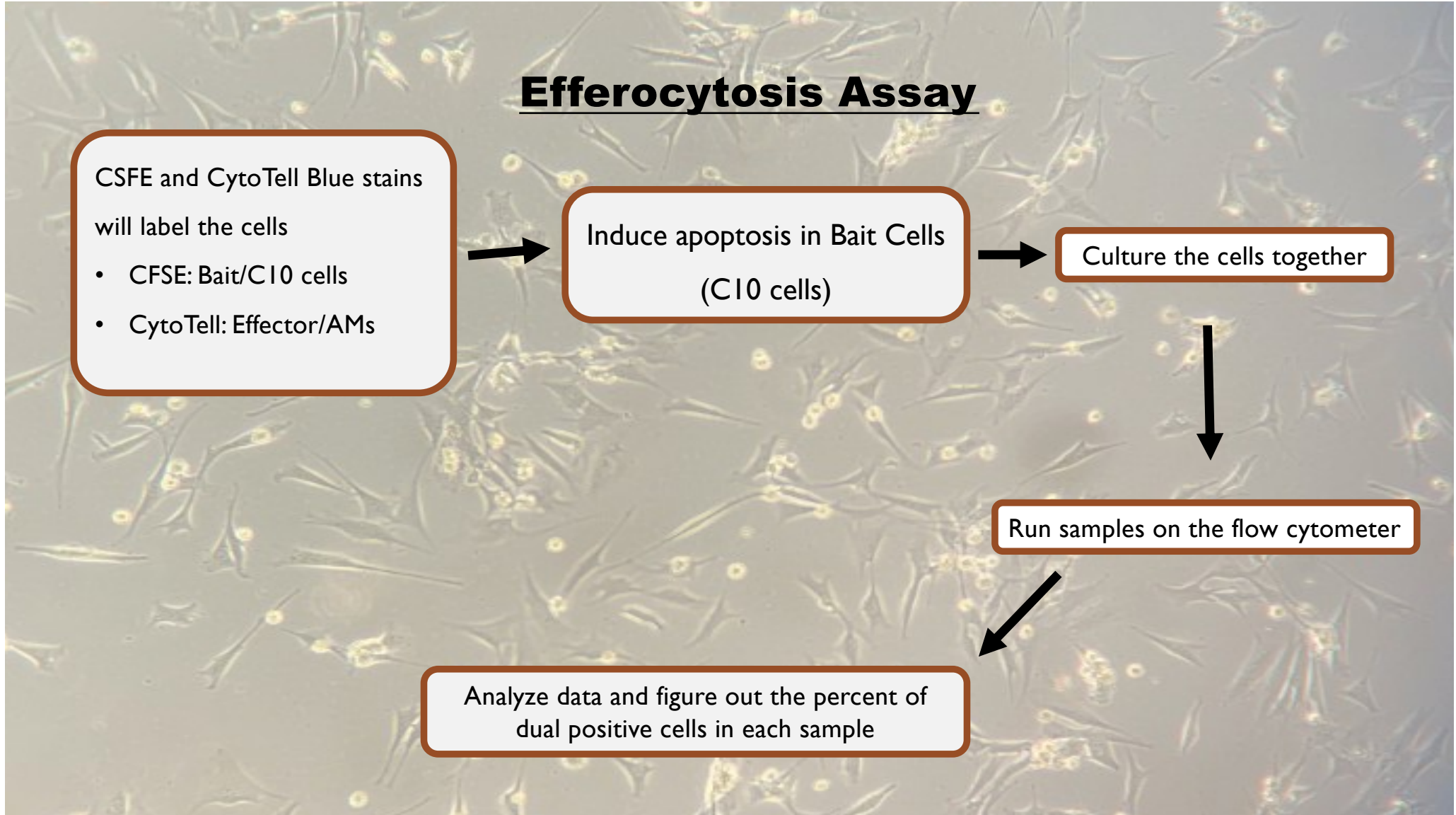
- CFSE: Bait/C10 cells
- CytoTell: Effector/AMs

Induce apoptosis in Bait Cells
(C10 cells)

Culture the cells together

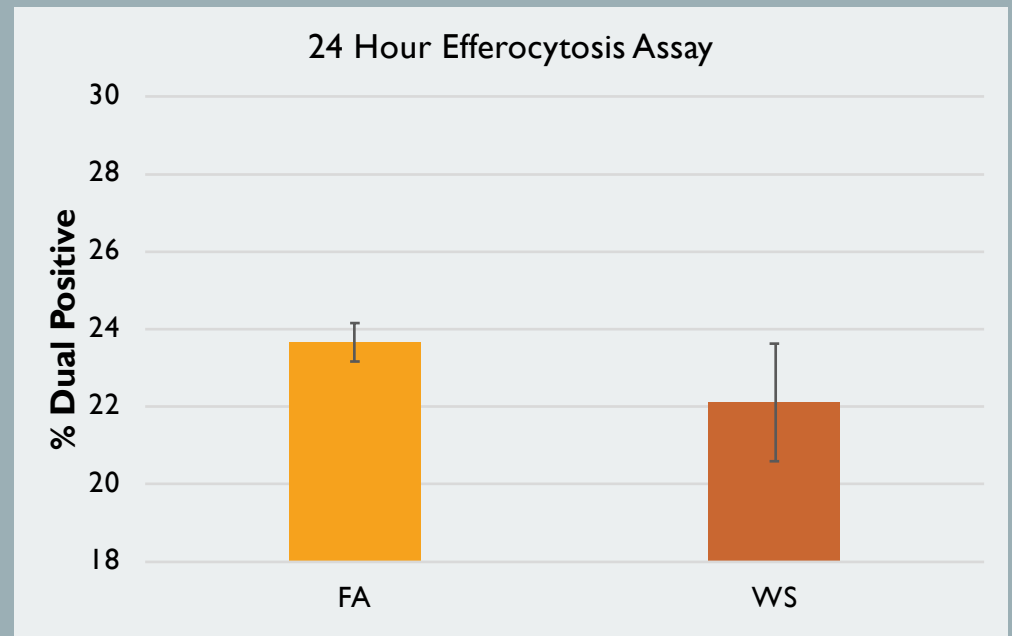
Run samples on the flow cytometer

Analyze data and figure out the percent of
dual positive cells in each sample



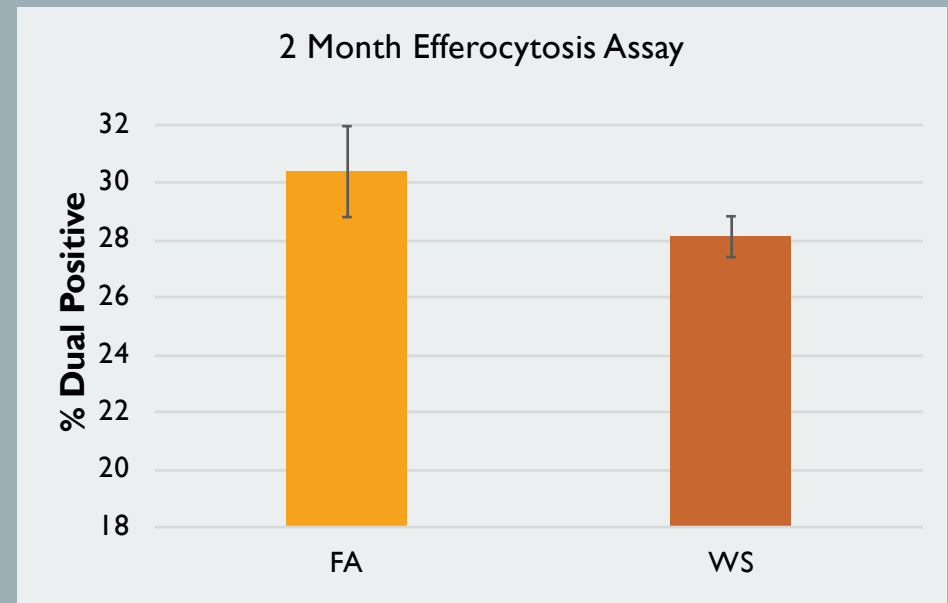
EFFEROCYTOSIS ASSAY: 24 HOUR POST EXPOSURE

- 12 total samples:
 - 5 filter air exposed mice
 - 7 wood smoke exposed mice
- Ran 1:2 effector:bait cell ratio
- Overnight

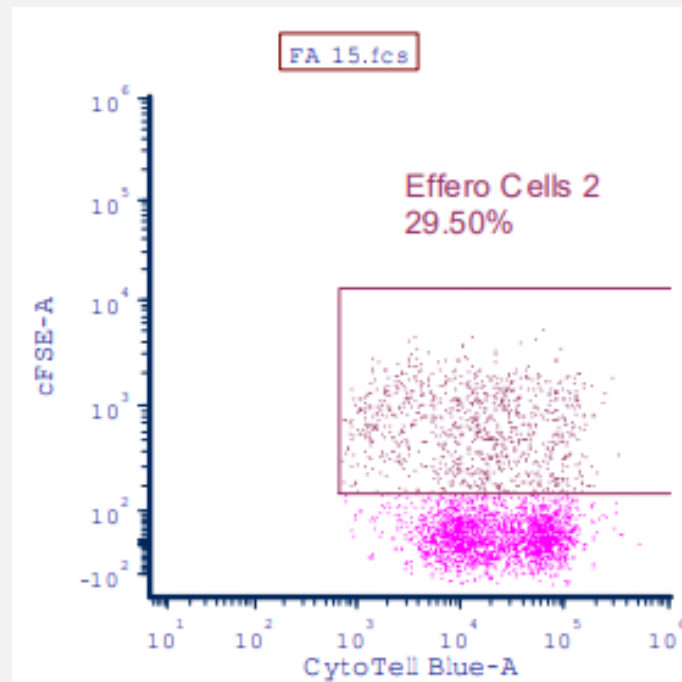


EFFEROCYTOSIS ASSAY: 2 MONTH POST EXPOSURE

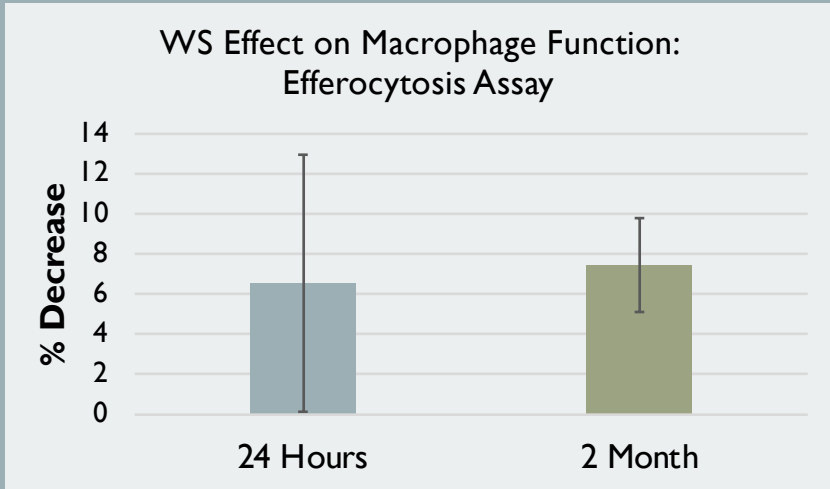
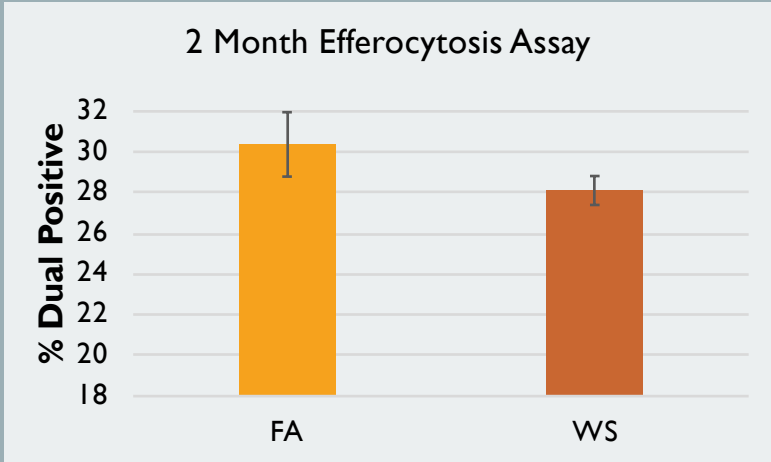
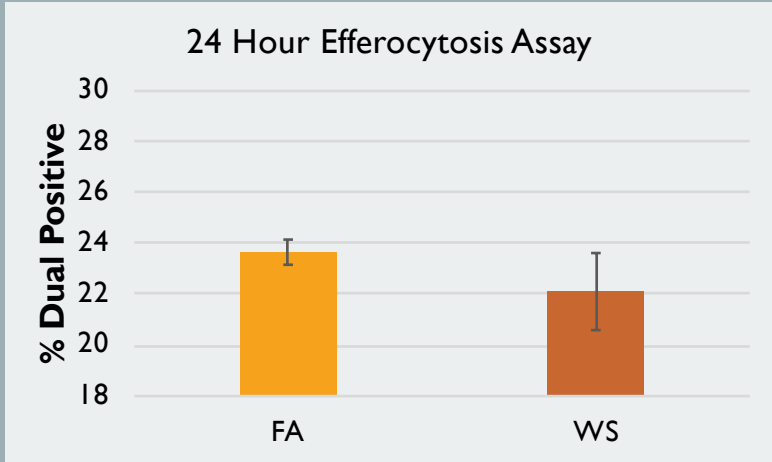
- 10 total samples:
 - 5 filter air exposed mice
 - 5 wood smoke exposed mice
- Ran 1:2 effector:bait cell ratio
- Overnight



EFFEROCYTOSIS ASSAY: FLOW CYTOMETRY GATING

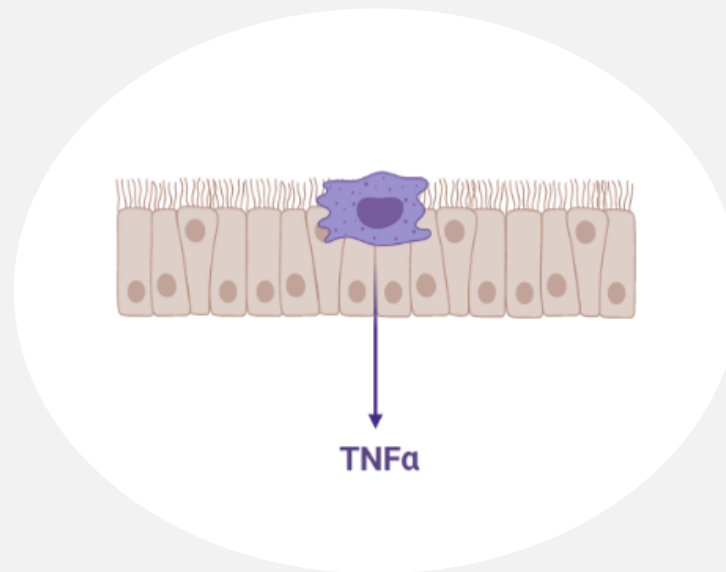


24 HOUR AND 2 MONTH DATA COMPARISON



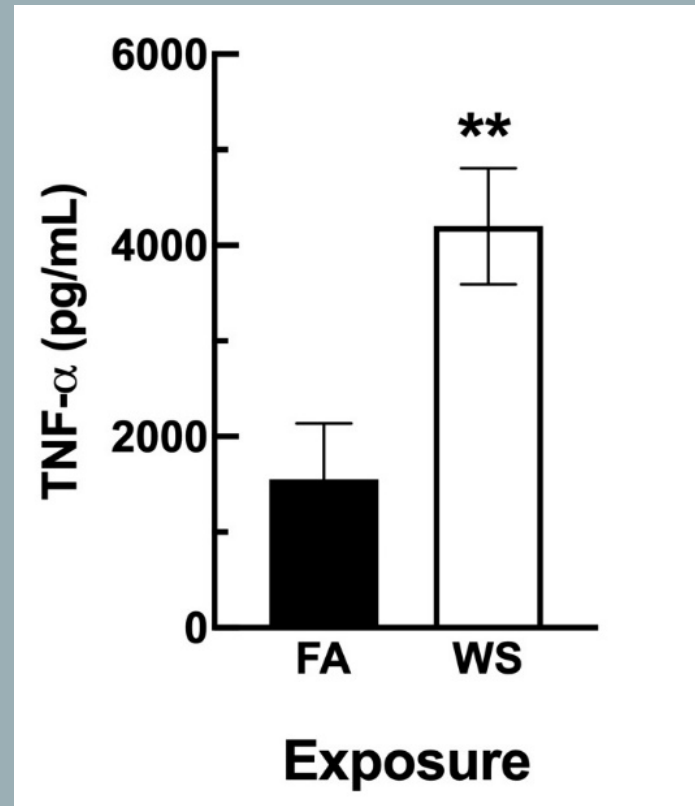
INFLAMMATORY RESPONSE: $\text{TNF}\alpha$

- Cytokine produced by macrophages during inflammation
- Promotes cell signaling which in turn leads to apoptosis (Idriss and Naismith 2000)



24 HOUR EXPOSURE: ELISA RESULTS

- Samples were exposed ex vivo to LPS
- TNF α showed a significant increase in mice exposed to wood smoke

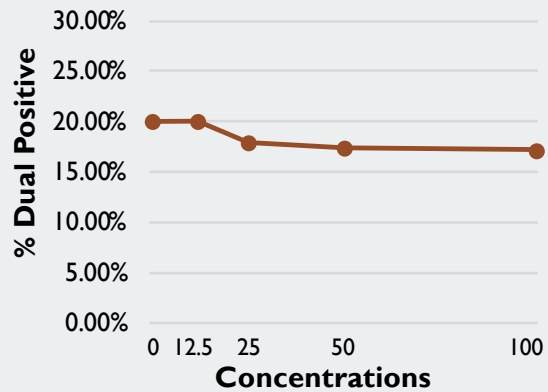


EFFEROCYTOSIS ASSAY: CRYSTALLINE SILICA AND BMDM

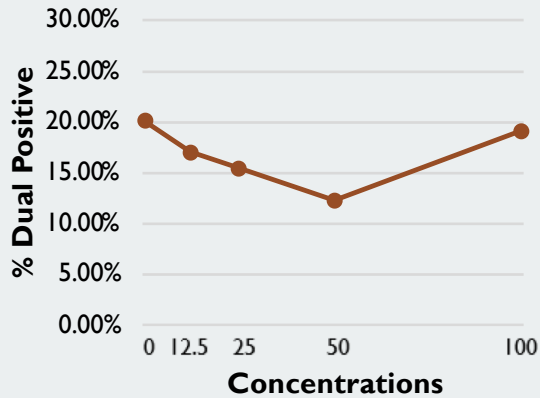
- 24 Samples:
 - 4: Min-U-Sil 5
 - 4: gQ tube #3 (synthetic quarts)
 - 4: gQ-f tube #4 (ball milled)
 - 4: vS (vitreous silica)
 - 4: gQ-f (high SSA) (milled silica)
- Concentrations:
 - 100, 50, 25, 12.5

CRYSTALLINE SILICA AND BMDM EFFEROCYTOSIS RESULTS

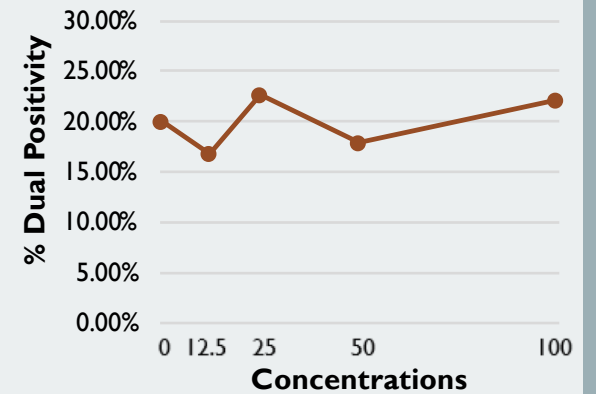
S1: min-u-sil 5



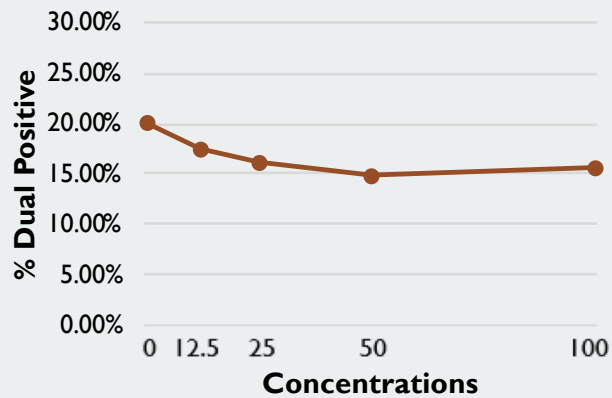
S2: gQ



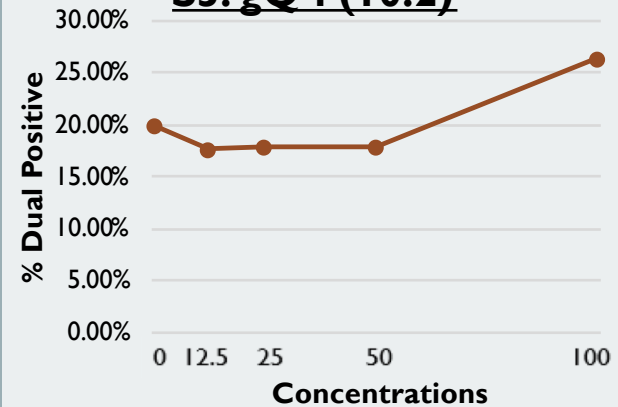
S3: gQ-f (3.2)



S4: Vitreous

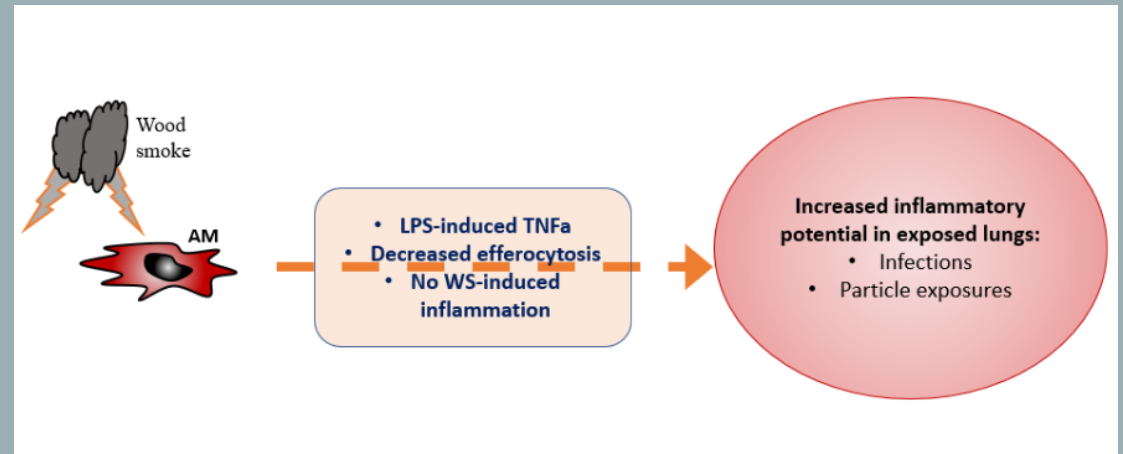


S5: gQ-f (10.2)



CONCLUSION

- Exposure to wood smoke increased inflammatory potential of exposed lungs
 - Decrease efferocytosis
 - Increase in TNF α
 - No neutrophil influx
- Results of the silica exposure shows that it is not a particle specific effect



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