



3-dimensional *in vitro* Models to Predict Relevant Toxicity or Pharmacological Effect in Human Lung

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Respiratory Toxicology Program



Agenda

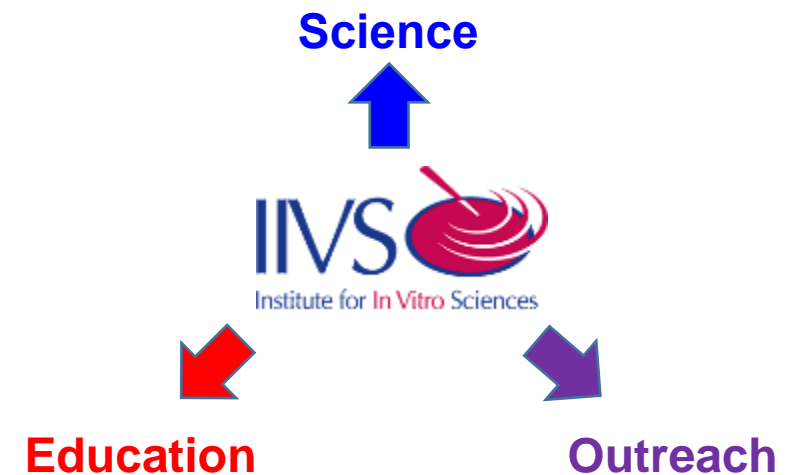
- IIVS: Who we are and What we do
- Inhalation Exposures & Methods
- Test Systems & Endpoints
 - Reconstituted Human Airways
 - Human Precision-cut Lung slices (hPCLS)
- Advancing hPCLS
 - Long term culture
 - Cryopreservation & Retained Functionality



Institute for In Vitro Sciences

- IIVS was founded as a **non-profit** laboratory in 1997 to promote the use and acceptance of *in vitro* methods for toxicology
- We provide testing services to a variety of industries including cosmetic, household product, specialty chemical and pesticide manufacturers (fee-for-service)
- We use our knowledge to create education and outreach programs to promote the regulatory acceptance of these methods worldwide (contributions)

We are regarded as an **independent technical authority** by industry, animal protection and governments around the world



A Variety of Inhaled Materials

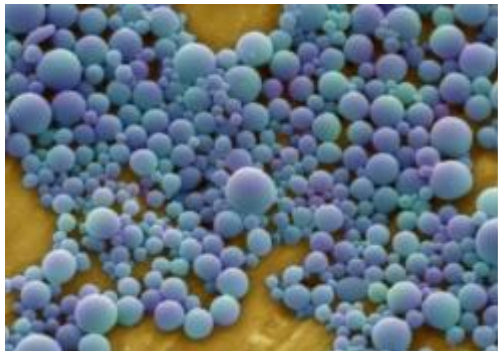
- Household products
 - cleaning agents, air care, upholstery and carpet care, “pesticides”
- Personal care products
 - sprays, powders, fragrances
- Occupational
 - institutional cleaning and care products, reactives, intermediates, volatiles
- Environmental
 - Smog, wildfire smoke, ozone
- Systemic exposures
 - Off target pharmaceutical effects



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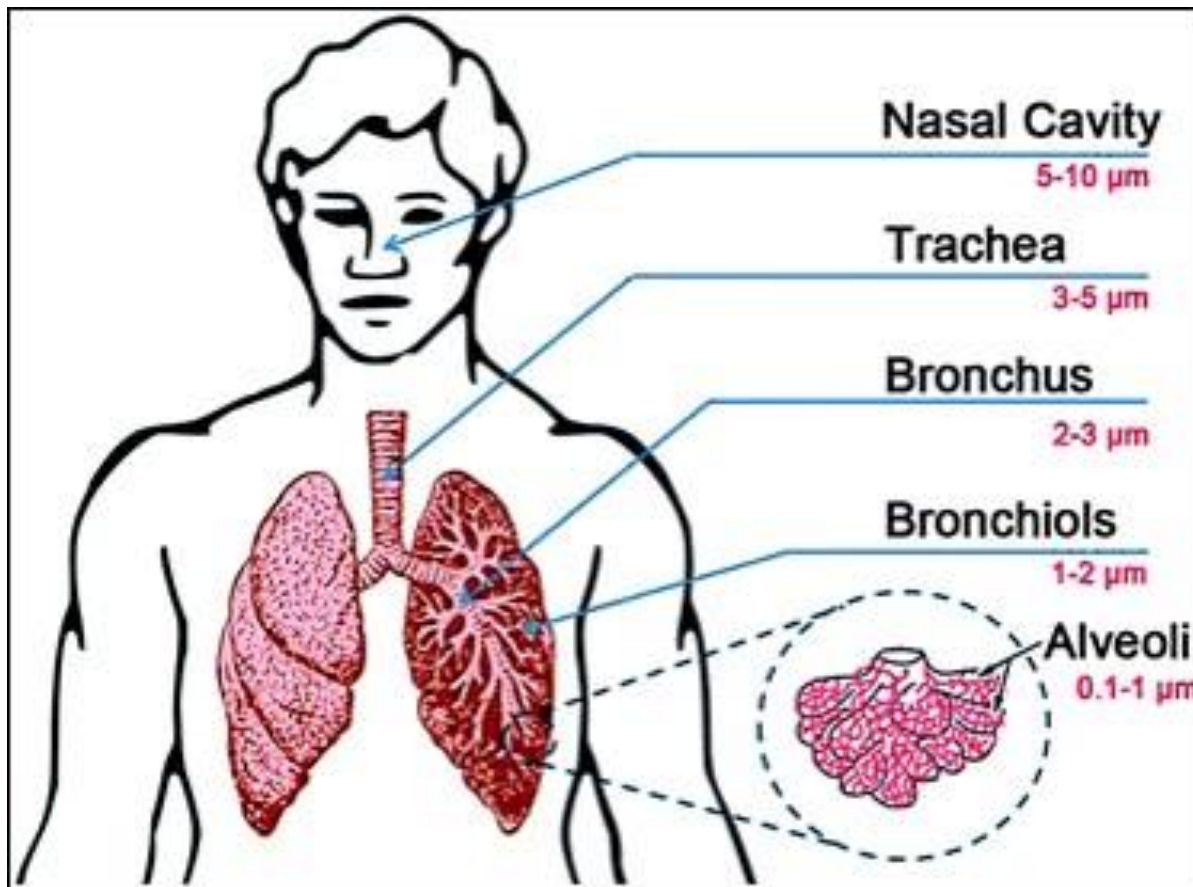


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Inhalation Exposure Complexity

Deposition of materials: Particle size and distribution

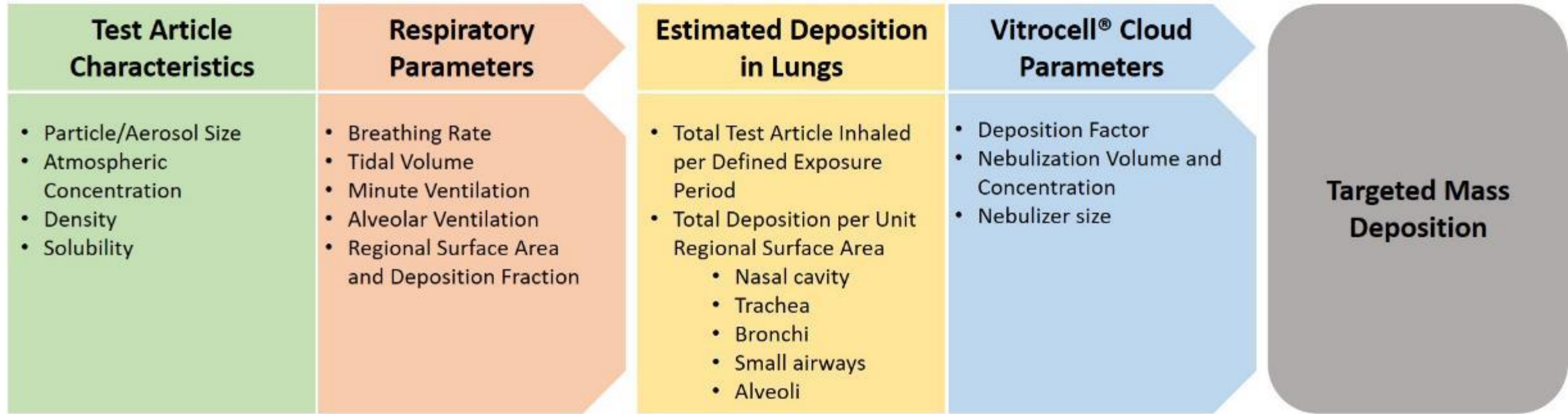


Deposition mechanisms:

- diffusion
- sedimentation
- inertial impaction
- interception
- electrostatic forces

all play a major or minor role

Target Mass Deposition Estimates



Additional Considerations:

- Mucociliary clearance
- Metabolism
- Diffusion

Estimated Total Deposition			
Parameters	1 hr	4 hr	8 hr
Minute ventilation (L)	444	1776	3552
Surface area deposited (cm ²)	14.69	14.69	14.69
Deposition fraction	0.13	0.13	0.13
TA concentration in air (µg/L)	87.10	34.84	21.78
Total TA inhaled (µg)	38672	61876	77345
Total TA Deposition (µg/cm²)	335.01	536.01	670.01

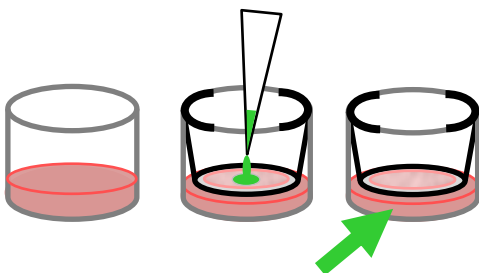
References Applied (select):

- Corley et al., 2012 (CFD)
- Corley et al., 2018 (EPA report - Chlorothalonil)
- Asgharian et al., 2012 (MPPD)
- EPA, 2004 (Air Quality PM)

Exposures: Aqueous, Digital, & Aerosols

Aqueous

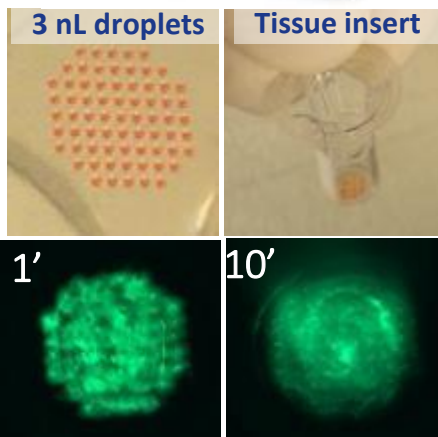
Conventional pipetting



- Material is solubilized or suspended in an aqueous medium or buffer
- Cons: non-aqueous soluble materials

Digital

Hewlett-Packard D300



- Biopattern dispensing: Entire exposure patterns can be **nanoliters** in volume
- Cons: Unique, emerging technology

Aerosol

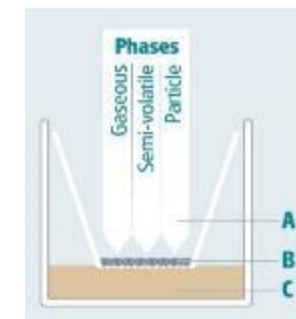
VITROCELL® Cloud12



VITROCELL® VC1

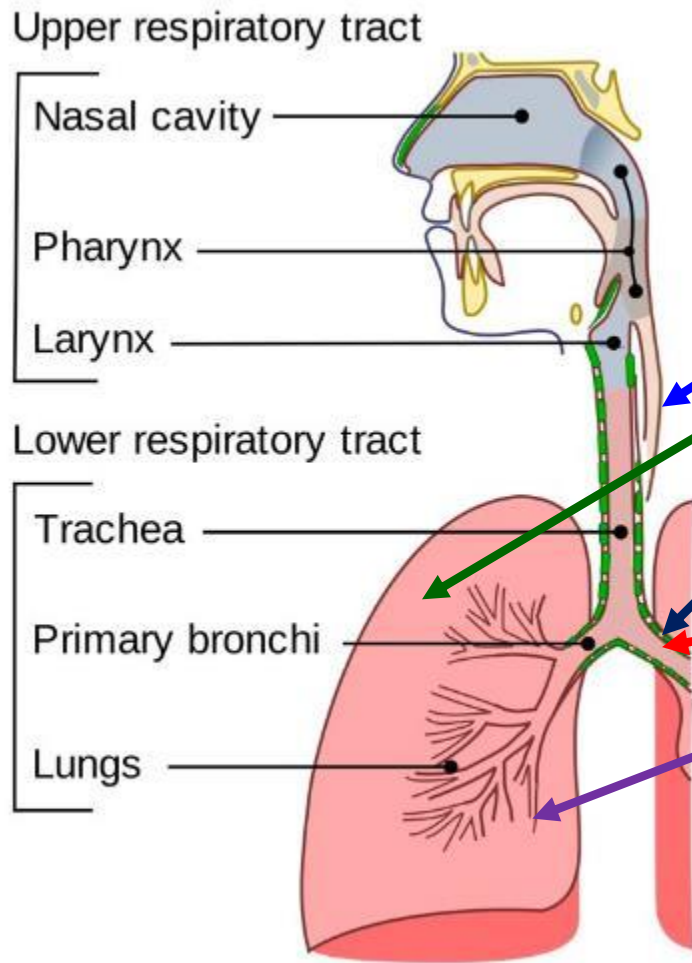


- Non-combusted & combusted aerosol generation
- Dosimetry
 - QCM
 - Surrogate analyte



- Cons: non-aqueous or miscible materials
- Setup/maintenance
- More labor intensive

2D Respiratory Cell Cultures



Numerous Primary Cells and Cell Lines

Cell lines

- H292 (muco-epidermoid carcinoma)
- A549 (alveolar basal epithelial cells)
- BEAS 2B (normal human bronchial epithelium, immortalized)

Primary Cells

- Normal Human Bronchial Epithelial Cells (NHBE)
- Normal Human Small Airway Epithelial Cells (NHSAE)

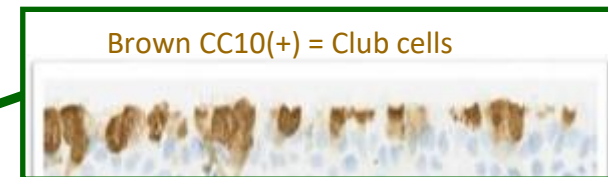
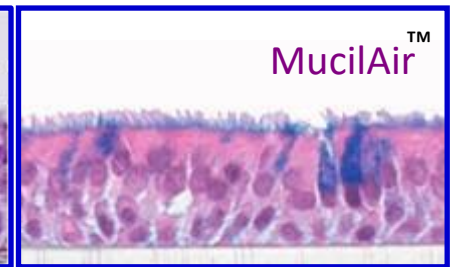
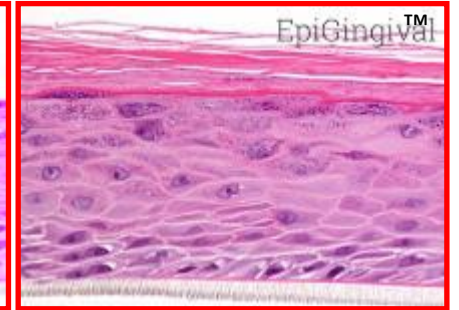
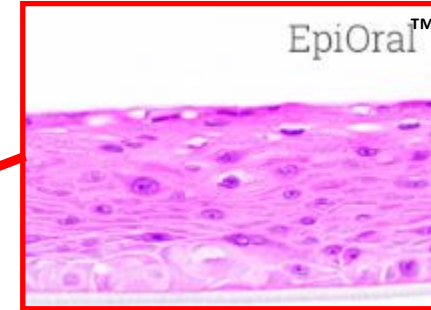
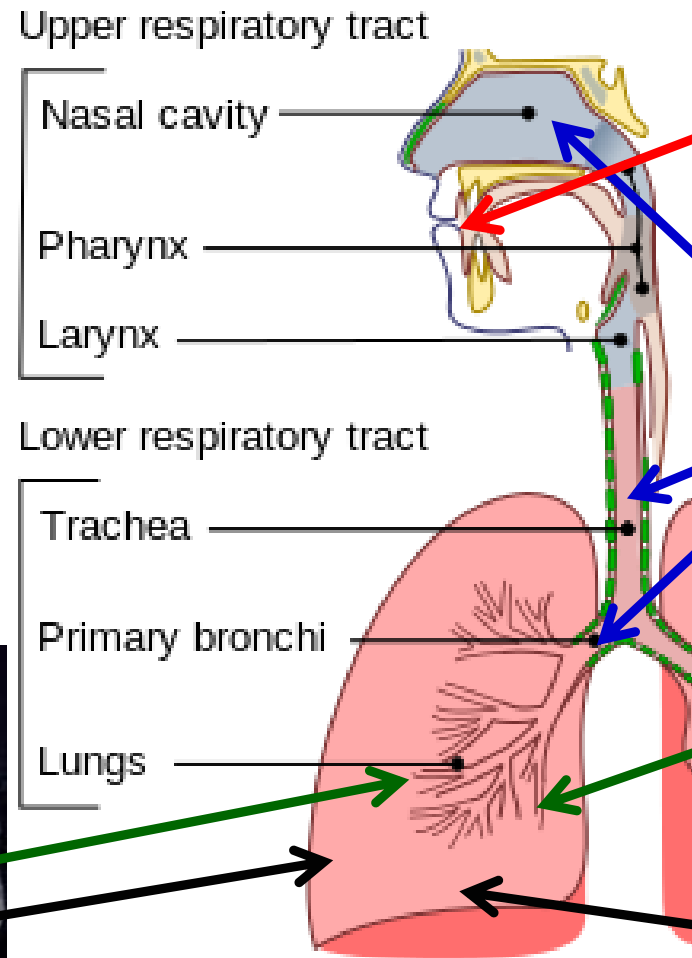
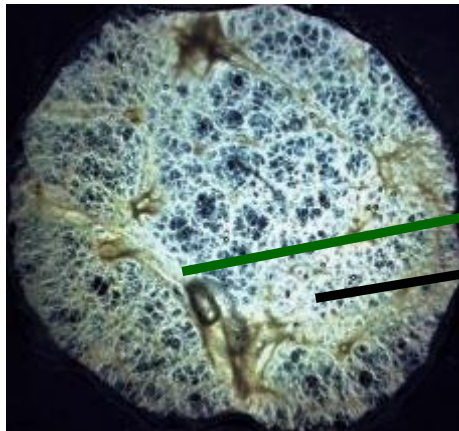
Pros: Higher throughput, more economical than 3D, useful for screening strategy. Lines can be maintained long-term with consistent results.

Cons: Primary cells are short live have a limited donor supply, but are more “normal-like” than lines. Lines are cancerous or immortalized, lacking normal attributes.

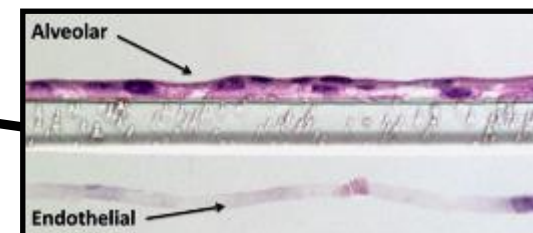
3D Respiratory Tract Models

Reconstructed Human Airways (RHuA)

Precision-cut Lung Slices (PCLS)

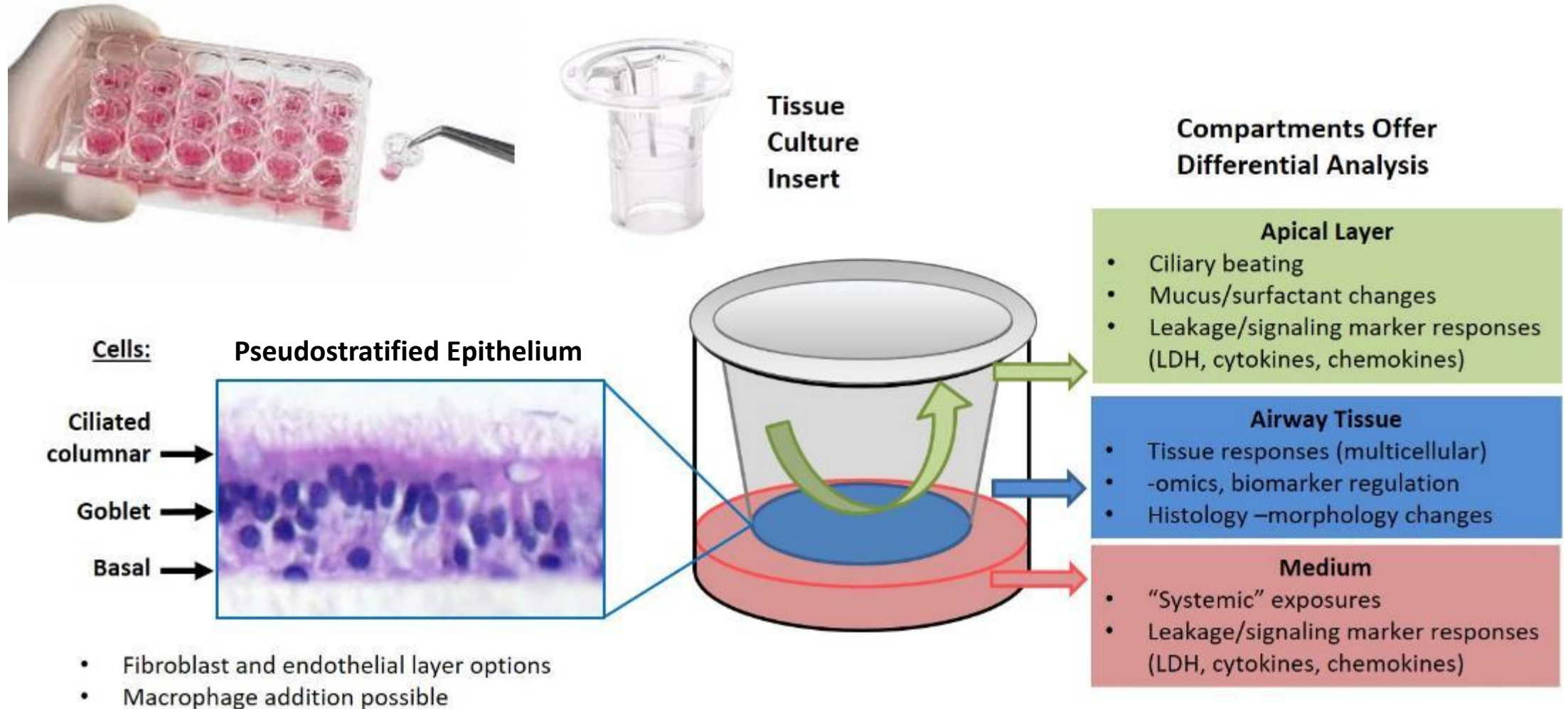


SmallAir™



EpiAlveolar™

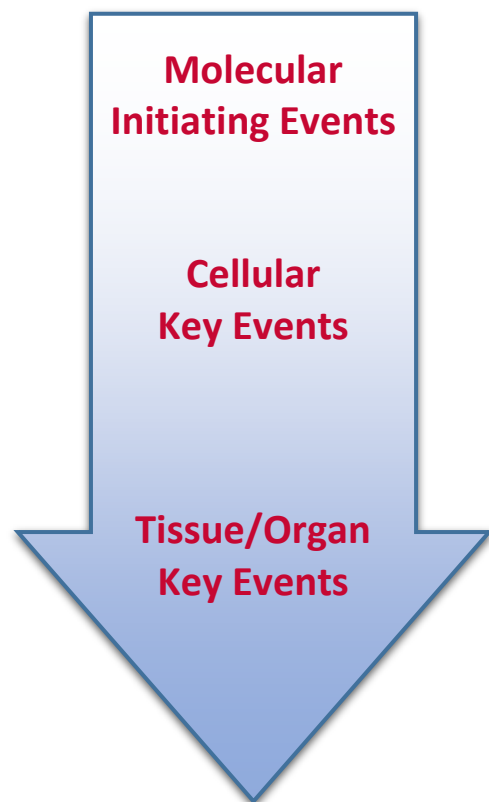
Reconstructed Human Airways (RHuA)

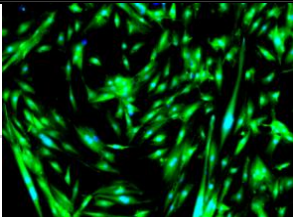
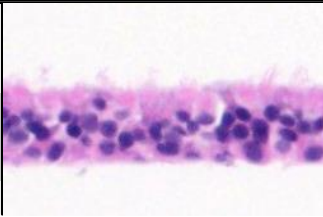
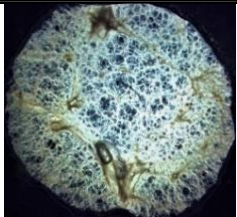


Pros: 3D epithelium & multiple cell types. RHuA offer airway “lumen”, mucous layer, & beating cilia. Long term cultures possible.

Cons: Closed system and no recruitment/systemic response. RHuA offer fewer cell types. Expensive & multi-week creation.

Choice of Test System & Endpoints



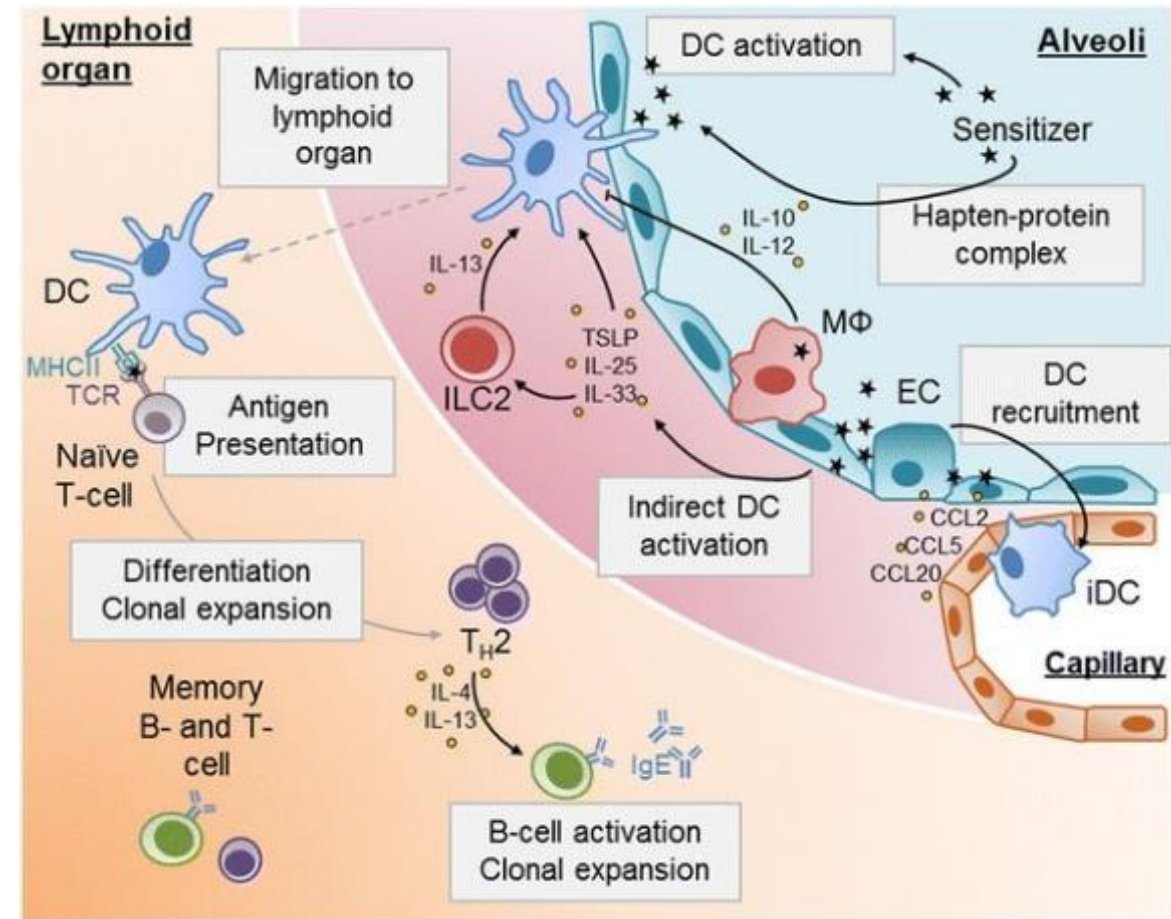
Exposure Aqueous - Solvent - Aerosol			
Test System			
Biomarkers/Events	2D Cells	3D RHuA	3D PCLS
Oxidative Stress	✓	✓	✓
DNA Binding/Strand Breaks	✓	✓	✓
Mitotoxicity	✓	✓	
Cytotoxicity	✓	✓	✓
Viability	✓	✓	✓
Macrophage Activation			☑
Cytokine/Chemokine Response	✓	✓	✓
Tight Junction Integrity		☑	
ECM Deposition			☑
Mucociliary Clearance		☑	
Goblet Cell Increase		☑	
Mucin Expression		☑	
Chronic Inflammation		✓	✓
Chronic Tissue Health		✓	☑

☑ =
unique
to Model

IIVS & Respiratory NAMs

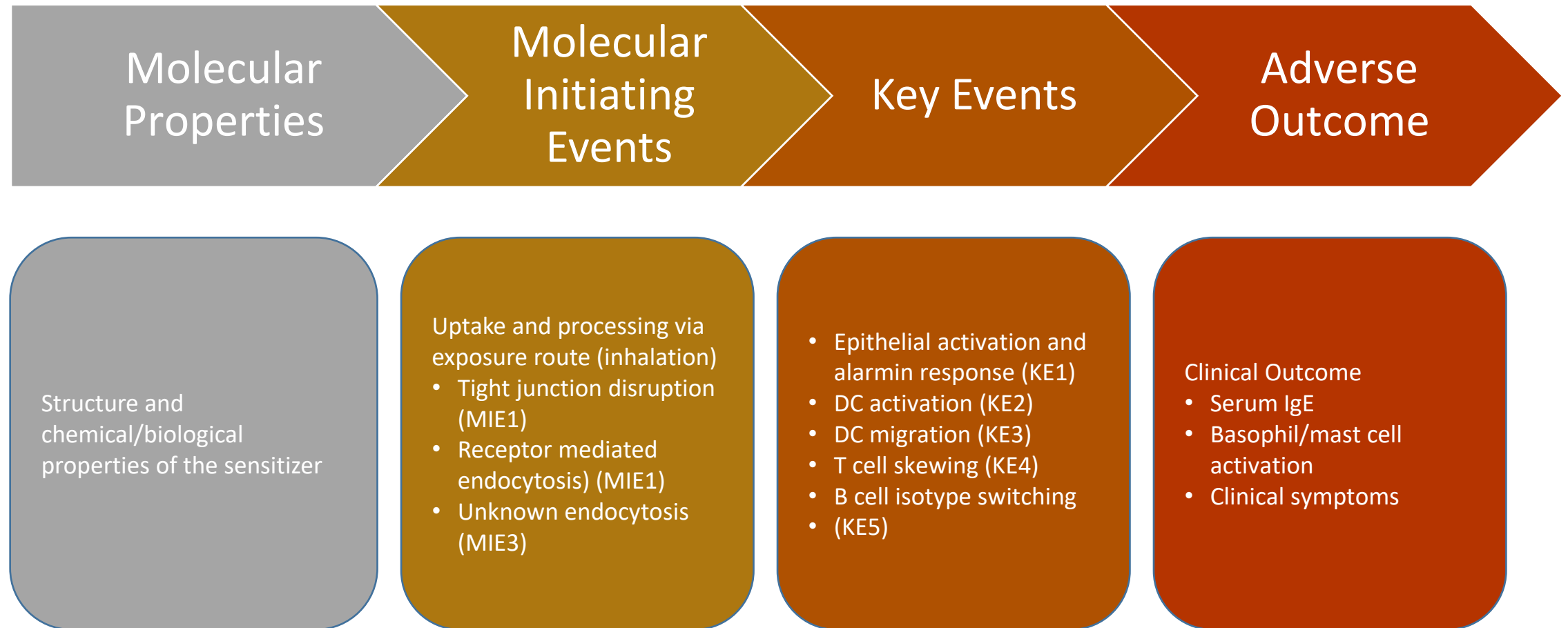
- IIVS was approached to **develop NAMs for evaluating fragrance materials** for respiratory irritation
- Respiratory Toxicology at IIVS has been active:
 - hosting workshops, participating in inter-lab technical exercises, presenting at international conferences, and serving on expert panels
- IIVS has been pursuing method development to address the need for identifying **respiratory sensitizers** and has recently formed a **collaboration with RIFM**

Respiratory Sensitization



Chary, A., Hennen, J., Klein, S.G. et al. Respiratory sensitization: toxicological point of view on the available assays. Arch Toxicol 92, 803–822 (2018).

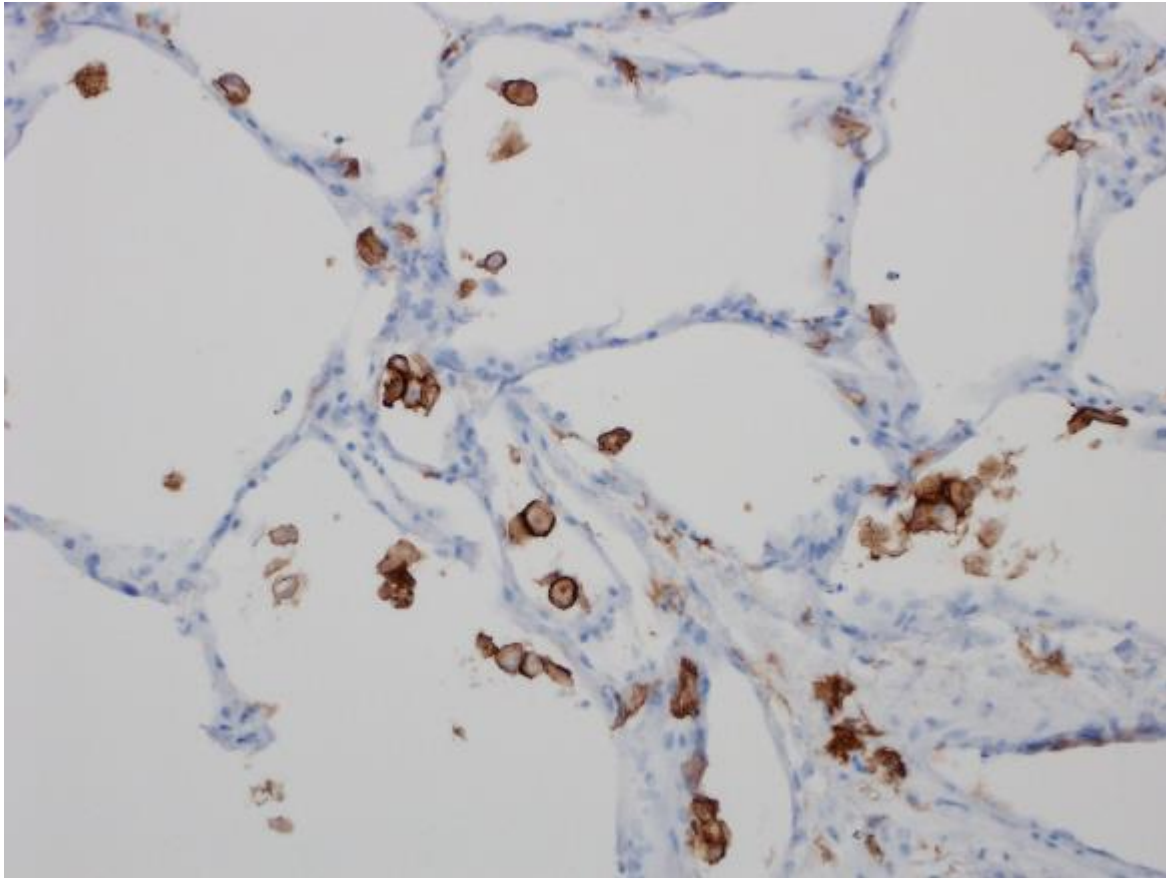
Respiratory Sensitization Adverse Outcome Pathway



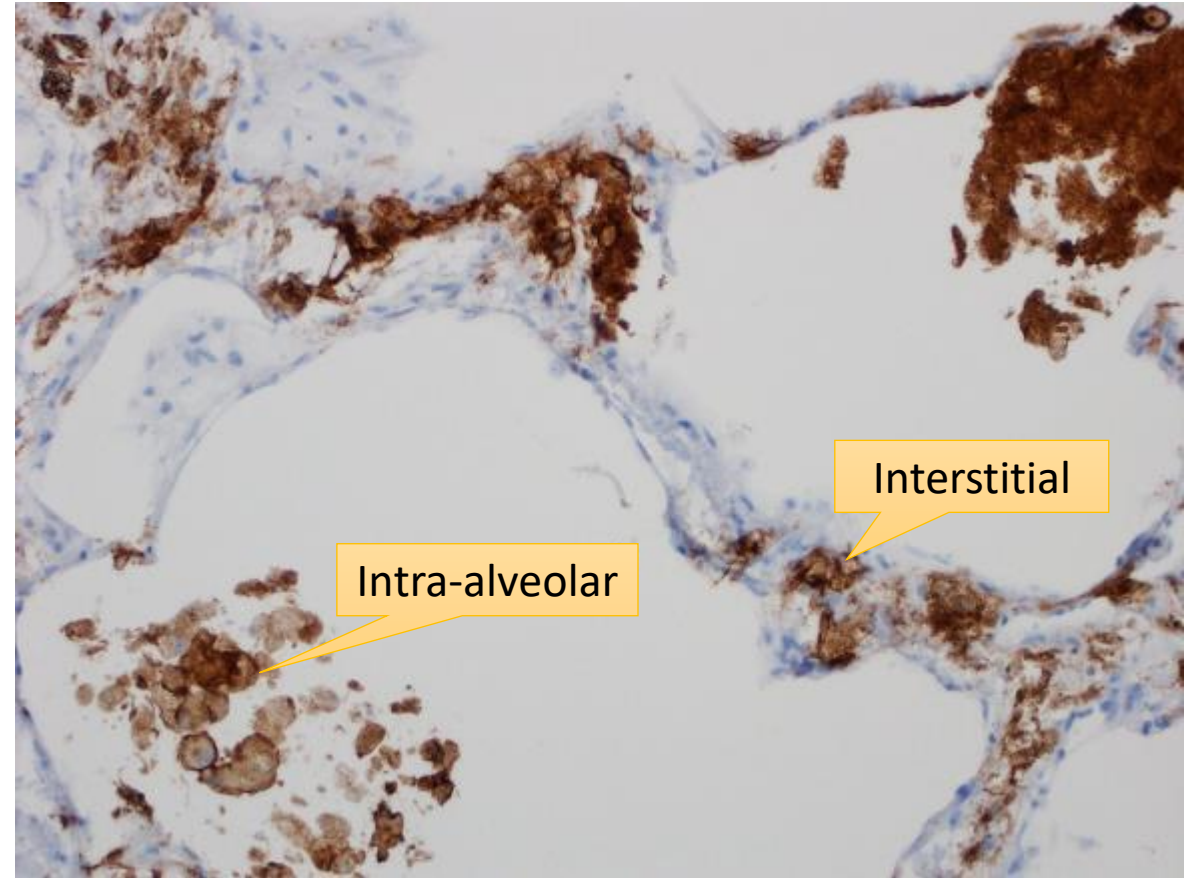
Based on van Bilsen et al, 2017, Sullivan et al, 2017 and Lozano-Ojalvo et al, 2019

PCLS: Activated Macrophages

Anti-CD86 IHC



Control (72hr)



5 µg/mL LPS (72hr)

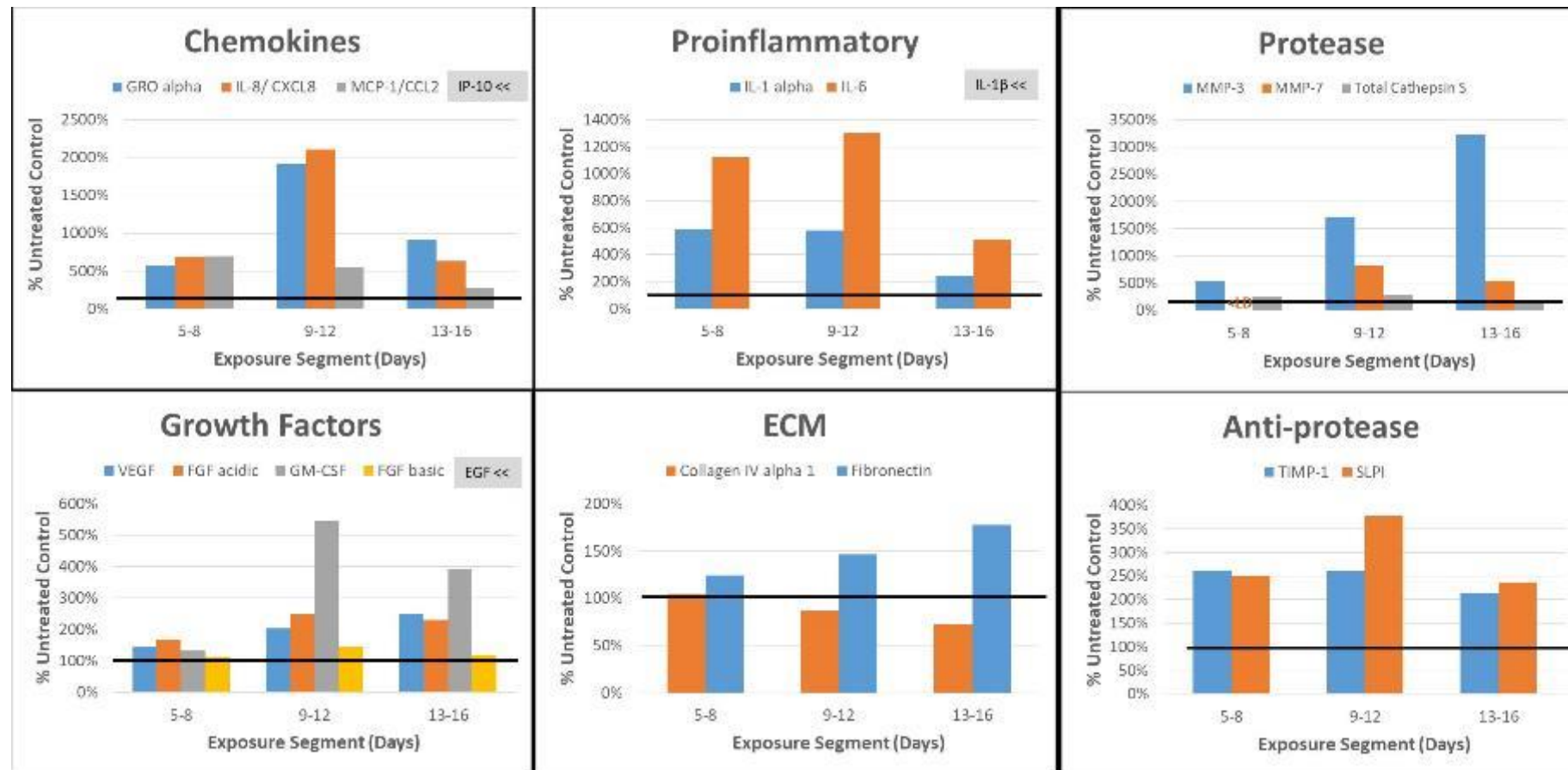
Multiplex Marker Upregulation

Quartz silica induces marker levels without loss of viability in PCLS



IIVS utilizes the **Luminex MagPix** and magnetic bead technology to multiplex and quantify marker levels from:

- Apical rinses
- Media
- Tissue lysates



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

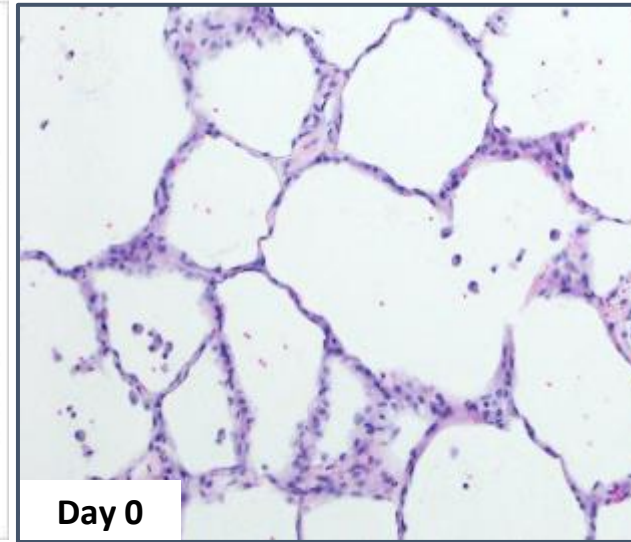
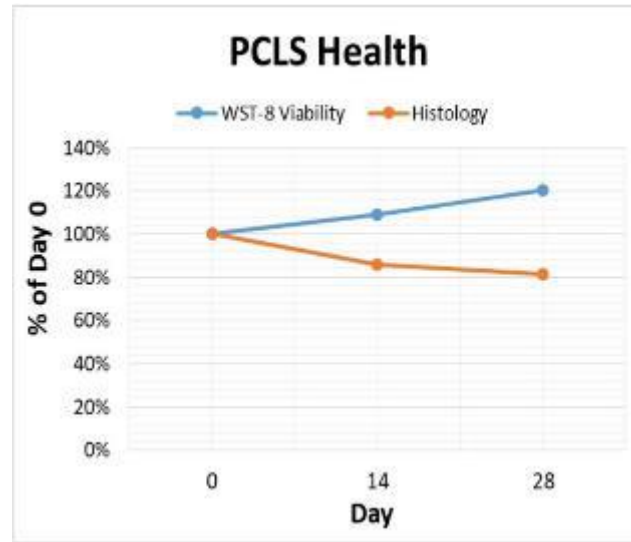


Advancing Human Precision-cut Lung Slices

Dr. Vivek Patel

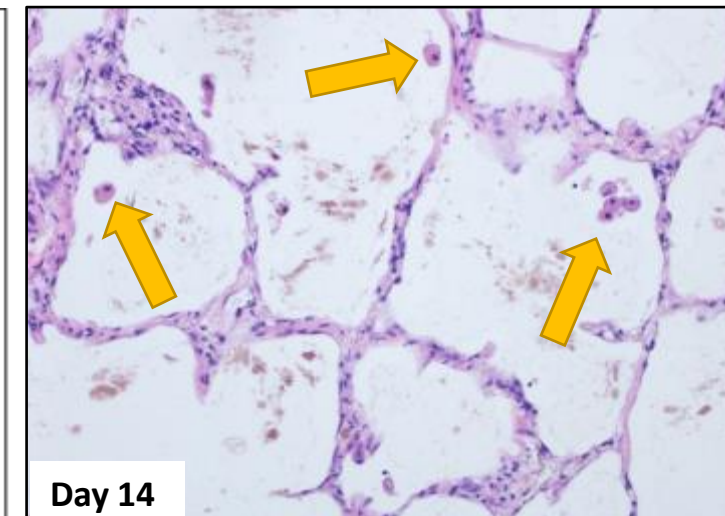
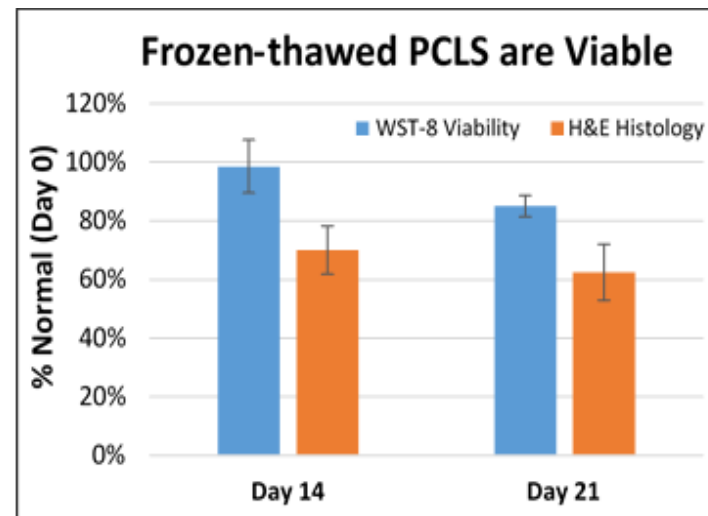
Long Term Human PCLS Culture

Fresh Human PCLS can be maintained for weeks to evaluate long term changes.

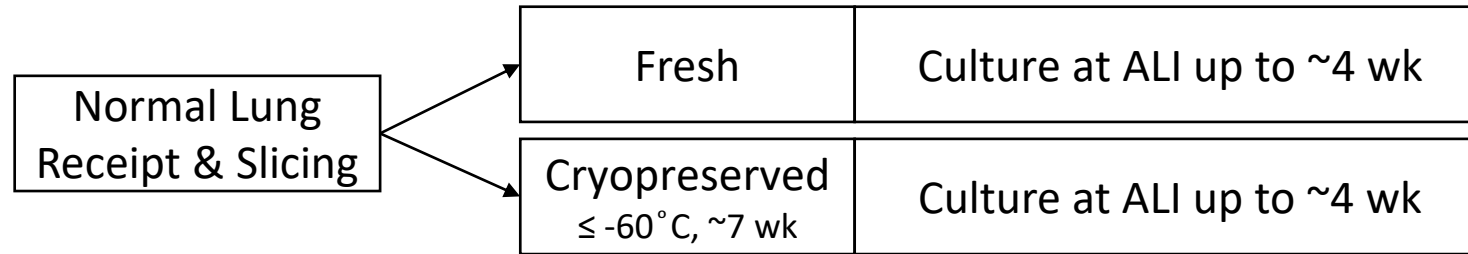


Frozen Human PCLS are now on demand

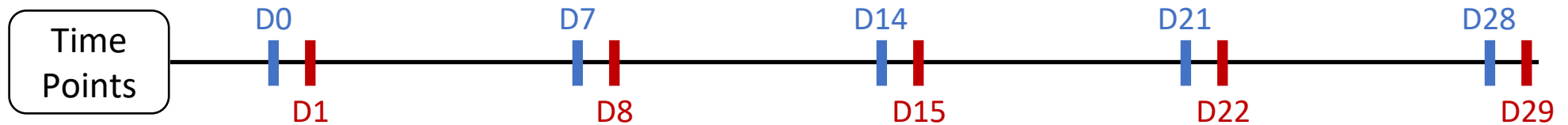
- IIVS developed cryopreservation buffer for longer post-thaw cultures
- Air-liquid interface (ALI) and submerged cultures compared
- Viable macrophages identified (arrows)



Characterization of cryopreserved PCLS

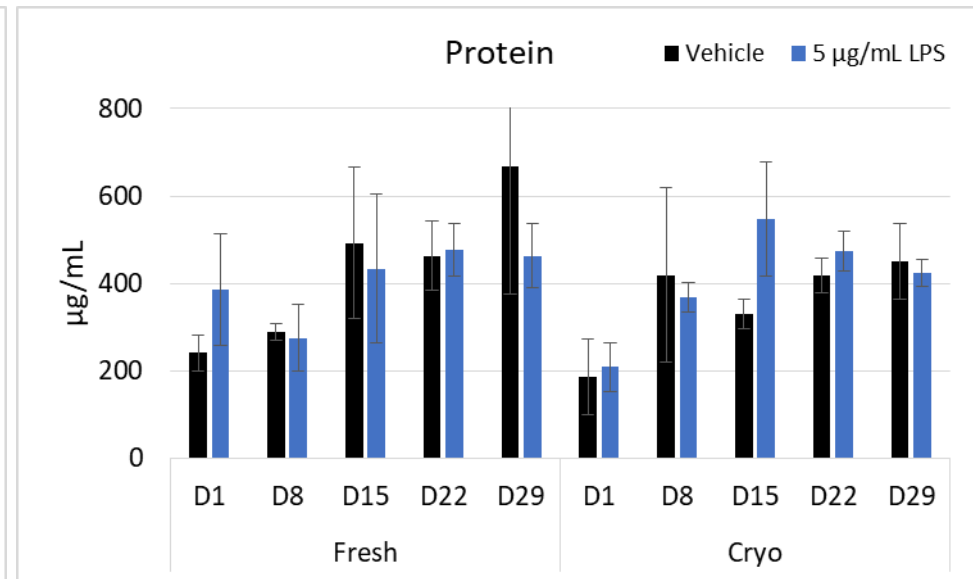
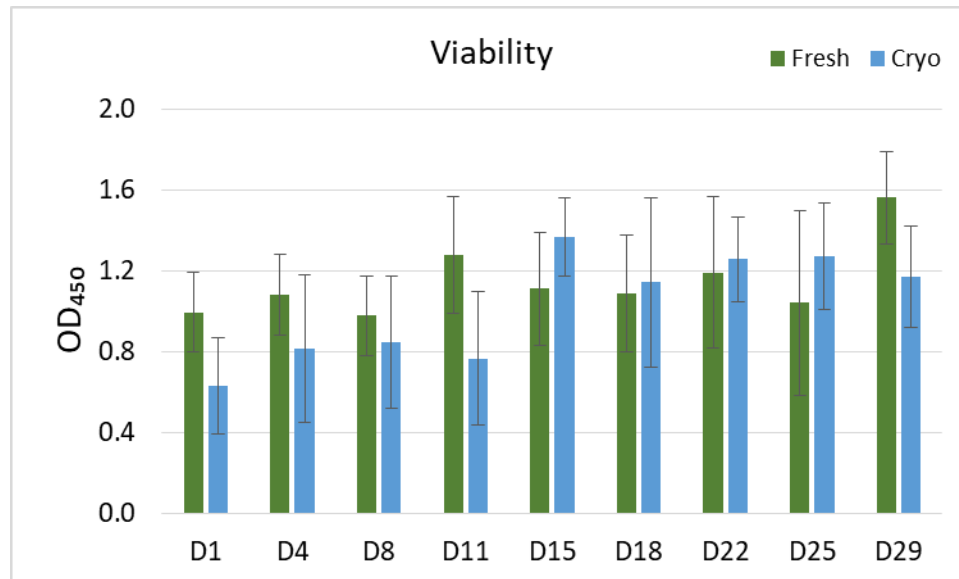
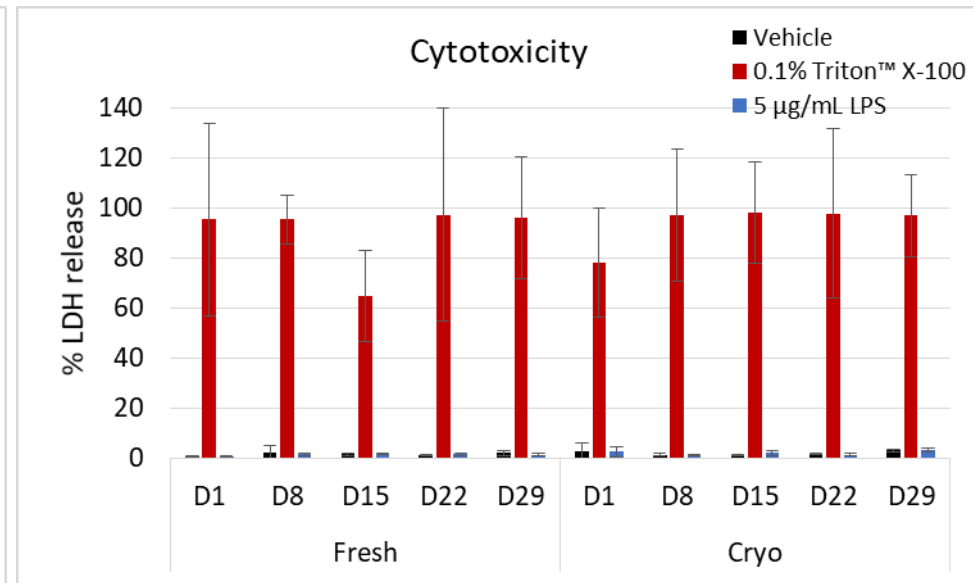
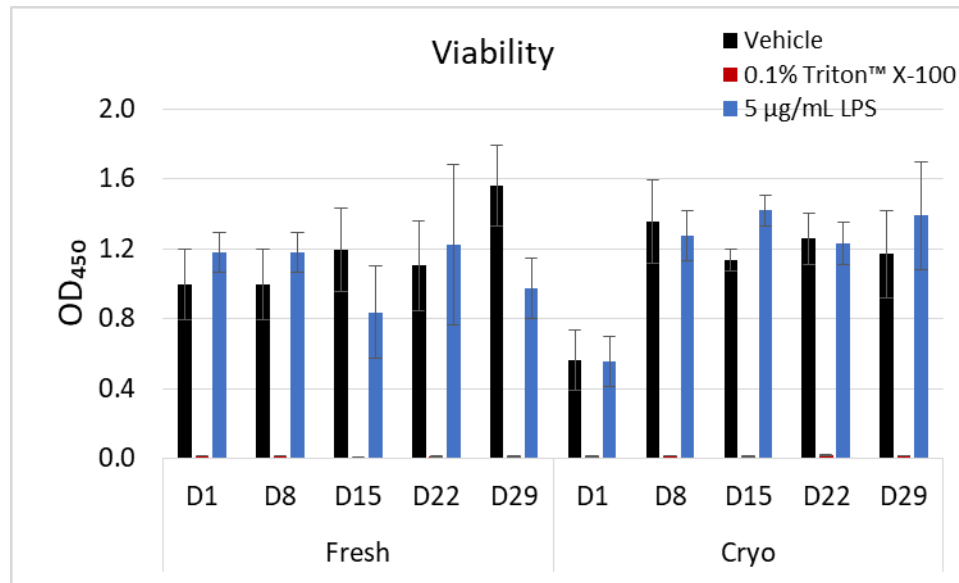


Treatment: Vehicle Control, 0.1% Triton™ X-100, 5 µg/mL LPS

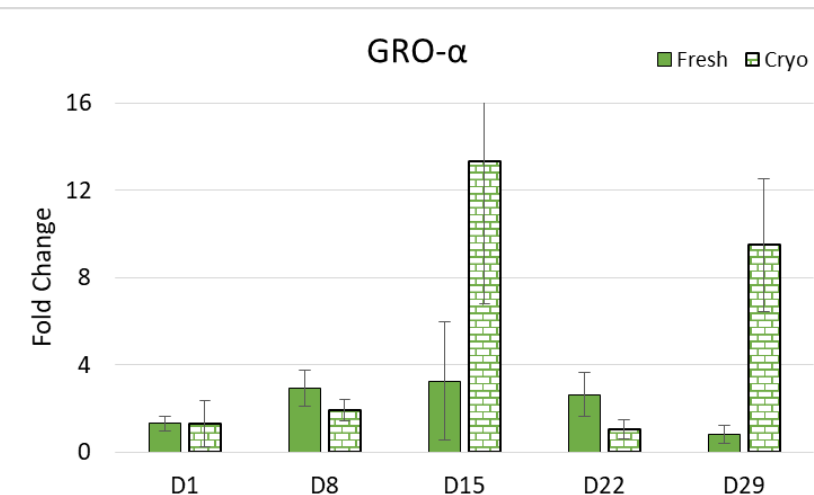
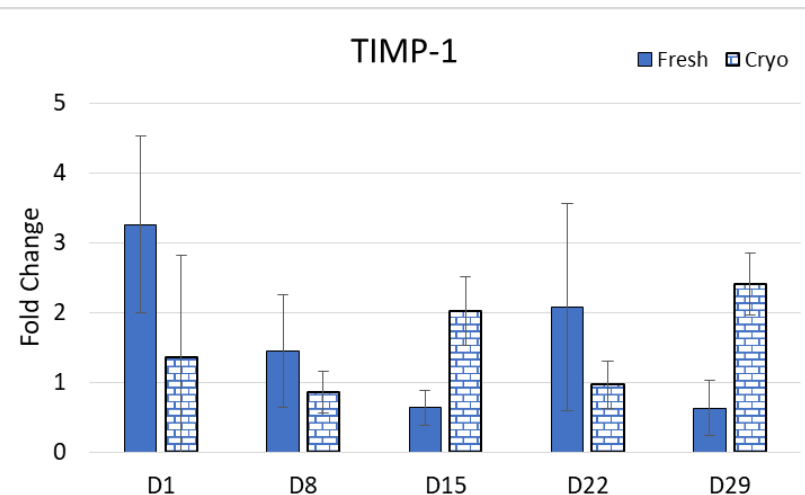
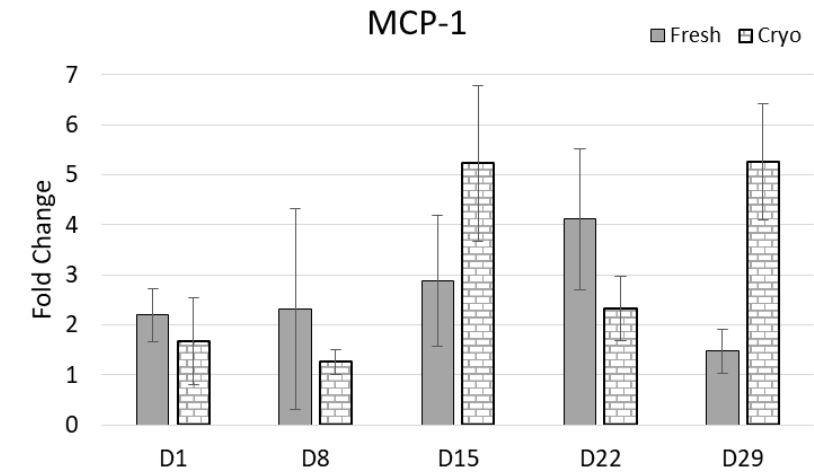
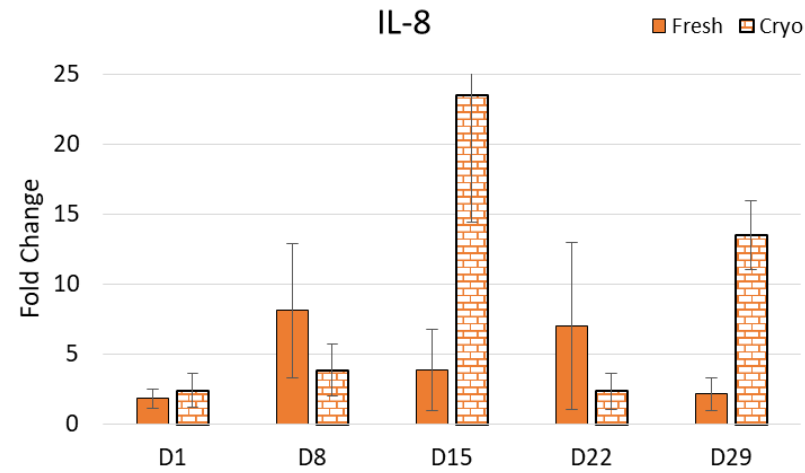
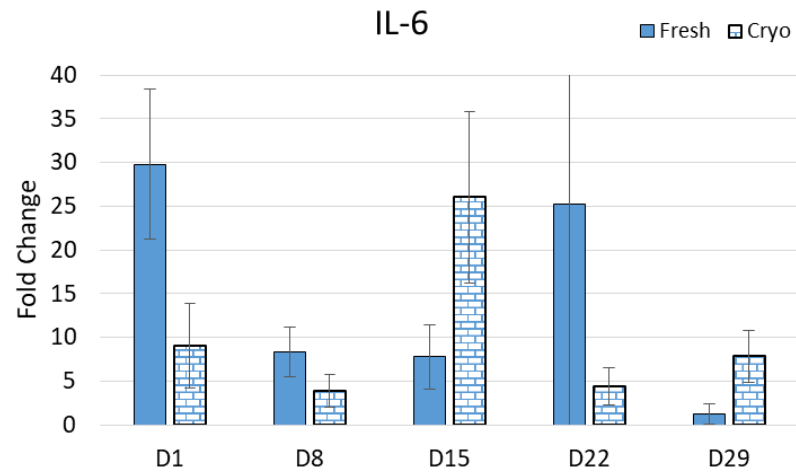


Endpoint Assessment: Viability (WST-8), Cytotoxicity (LDH),
Tissue response (Luminex Multiplex)

Triton™ X-100-induced Cytotoxicity



LPS-induced Tissue Responses



- LPS-induced IL-6, IL-8 and MCP-1 responses are observed through the 4 wk culture period
- Weak TIMP-1 and GRO- α response to LPS
- Efforts exploring consistency in responses are under way

Next Steps for hPCLS?

- Continue developing the application of hPCLS (fresh and frozen) for test material screening
 - Detection of sensitization vs irritation markers
 - Generation of the fibrosis phenotype
 - Evaluation of long-term effects (following single or repeat exposures)
 - Comparison of donor demographic-based responses
- Advocate standardization of hPCLS methodologies!
 - Consistent methods and data will position hPCLS as a candidate test system for regulatory testing applications

Acknowledgments

IIVS's staff, supporters, & collaborators!



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Khalid Amin, MD
(Pathologist, Assistant
Professor, Uni. Minn.)

*Thank
you*

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